

ATG GTA CGT AGC TCC TCT CGC ACT CCG TCC GAT AAG CCG GTT GCT  
 M V R S S S R T P S D K P V A  
 CAT GTA GTT GCT AAC CCT CAG GCA GAA GGT CAG CTG CAG TGG CTG  
 H V V A N P Q A E G Q L Q W L  
 AAC CGT CGC GCT AAC GCC CTG CTG GCA AAC GGC GTT GAG CTC CGT  
 N R R A N A L L A N G V E L R  
 GAT AAG CAG CTC GTG GTA CCT TCT GAA GGT CTG TAC CTG ATC TAT  
 D N Q L V V P S E G L Y L I Y  
 TCT CAA GTA CTG TTC AAG GGT CAG GGC TGC CCG TCG ACT CAT GTT  
 S Q V L F K G Q G C P S T H V  
 CTG CTG ACT CAC ACC ATC AGC CGT ATT GCT GTA TCT TAC CAG ACC  
 L L T H T I S R I A V S Y Q T  
 AAA GTT AAC CTG CTG AGC GCT ATC AAG TCT CCG TGC CAG CGT GAA  
 K V N L L S A I K S P C Q R E  
 ACT CCC GAG GGT GCA GAA GCG AAA CCA TGG TAT GAA CCG ATC TAC  
 T P E G A E A K P W Y E P I Y  
 CTG GGT GGC GTA TTT CAA CTG GAG AAA GGT GAC CGT CTG TCC GCA  
 L G G V F Q L E K G D R L S A  
 GAA ATC AAC CGT CCT GAC TAT CTA GAT TTC GCT GAA TCT GGC CAG  
 E I N R P D Y L D F A E S G Q  
 GTG TAC TTC GGT ATT ATC GCA CTG TAA  
 V Y F G I I A L \*

FIG. 1

## Derivation of the VNP20009(*serC*<sup>-</sup>) strain.

pCR2.1*serC*

(*serC* cloned by pcr into pCR2.1)

↓  
Clal+Eco47III restriction  
Mung bean nuclease degradation  
Religation  
Transformation into DH5α

pCR2.1Δ*serC*

(*serC* deletion cloned by pcr into pCR2.1)

↓  
Sac1+Xho1 restriction  
Isolation of 680 bp Δ*serC* gene  
Ligation into pCVD442  
Transformation into SM10 cells

pCVD442Δ*serC*

(*serC* deletion cloned into pCVD442 sucrase vector)

↓  
SM10 bacteria mated with *S. typhimurium*  
strain 501 to form merodiploid  
*serC* deletion transduced into VNP20009 using  
P22 bacteriophage  
VNP20009(*serC*<sup>-</sup>) obtained by sucrose selection

VNP20009 (*serC*<sup>-</sup>)

**FIG. 2**

## Quantitation of TNF $\alpha$ expression by pTS-BrpTNF $\alpha$ Clone 2.

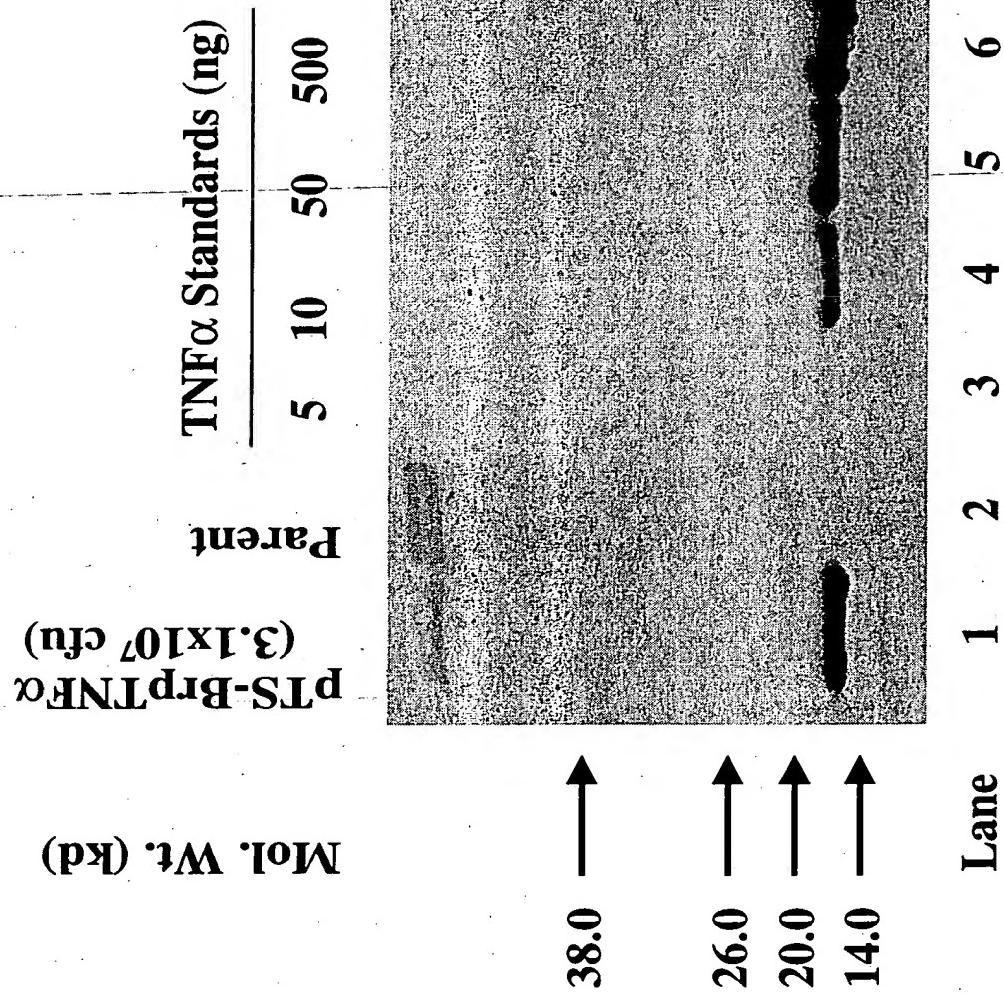


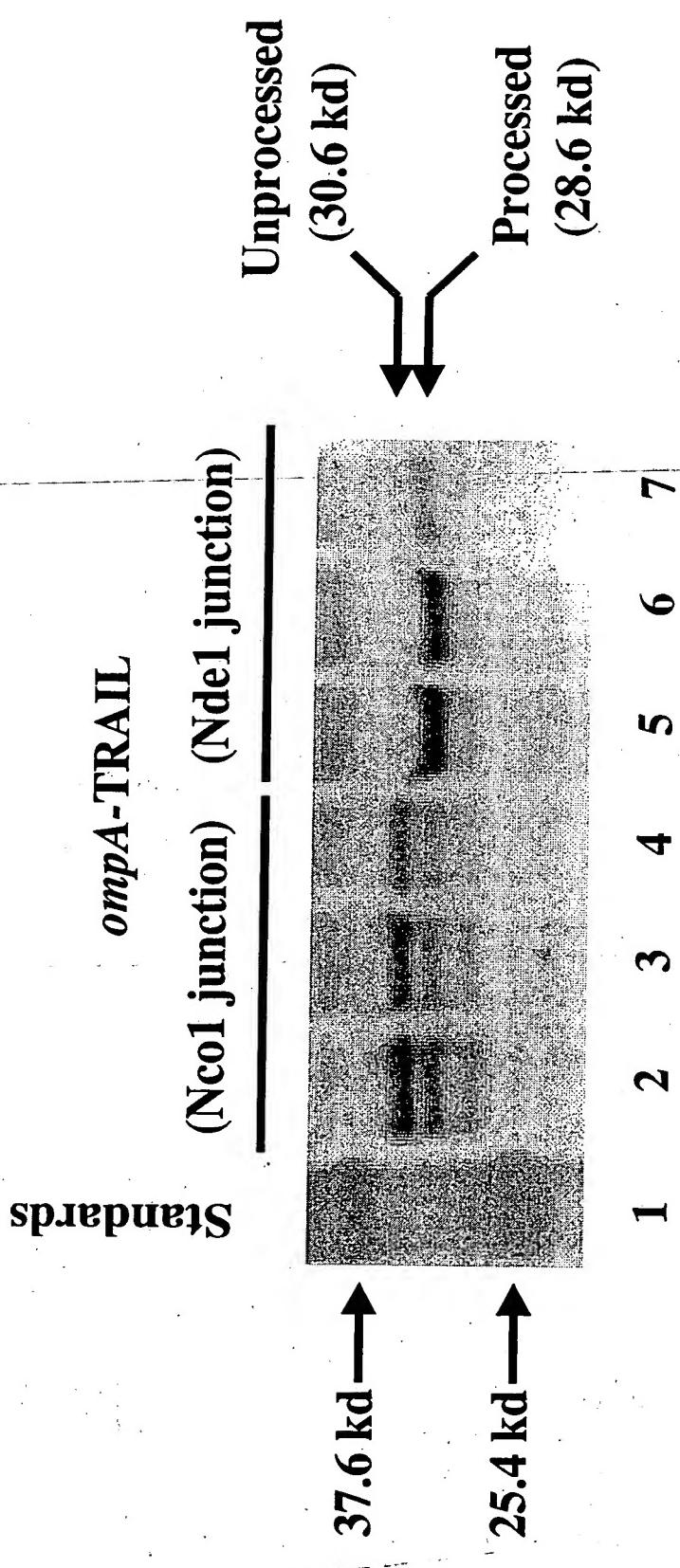
FIG. 3

ATG AAA AAG ACA GCT ATC GCG ATT GCA GTG GCA CTG GCT GGT TTC  
 M K K T A I A I A V A L A G F  
 GCT ACC GTA GCG CAG GCC CAT ATG GTA CGT AGC TCC TCT CGC ACT  
 A T V A Q A H M V R S S S R T  
 CCG TCC GAT AAG CCG GTT GCT CAT GTA GTT GCT AAC CCT CAG GCA  
 P S D K P V A H V V A N P Q A  
 GAA GGT CAG CTG CAG TGG CTG AAC CGT CGC GCT AAC GCC CTG CTG  
 E G Q L Q W L N R R A N A L L  
 GCA AAC GGC GTT GAG CTC CGT GAT AAC CAG CTC GTG GTA CCT TCT  
 A N G V E L R D N Q L V V P S  
 GAA GGT CTG TAC CTG ATC TAT TCT CAA GTA CTG TTC AAG GGT CAG  
 E G L Y L I Y S Q V L F K G Q  
 GGC TGC CCG TCG ACT CAT GTT CTG CTG ACT CAC ACC ATC AGC CGT  
 G C P S T H V L L T H T I S R  
 ATT GCT GTA TCT TAC CAG ACC AAA GTT AAC CTG CTG AGC GCT ATC  
 I A V S Y Q T K V N L L S A I  
 AAG TCT CCG TGC CAG CGT GAA ACT CCC GAG GGT GCA GAA GCG AAA  
 K S P C Q R E T P E G A E A K  
 CCA TGG TAT GAA CCG ATC TAC CTG GGT GGC GTA TTT CAA CTG GAG  
 P W Y E P I Y L G G V F Q L E  
 AAA GGT GAC CGT CTG TCC GCA GAA ATC AAC CGT CCT GAC TAT CTA  
 K G D R L S A E I N R P D Y L  
 GAT TTC GCT GAA TCT GGC CAG GTG TAC TTC GGT ATT ATC GCA CTG  
 D F A E S G Q V Y F G I I A L

TAA

\*

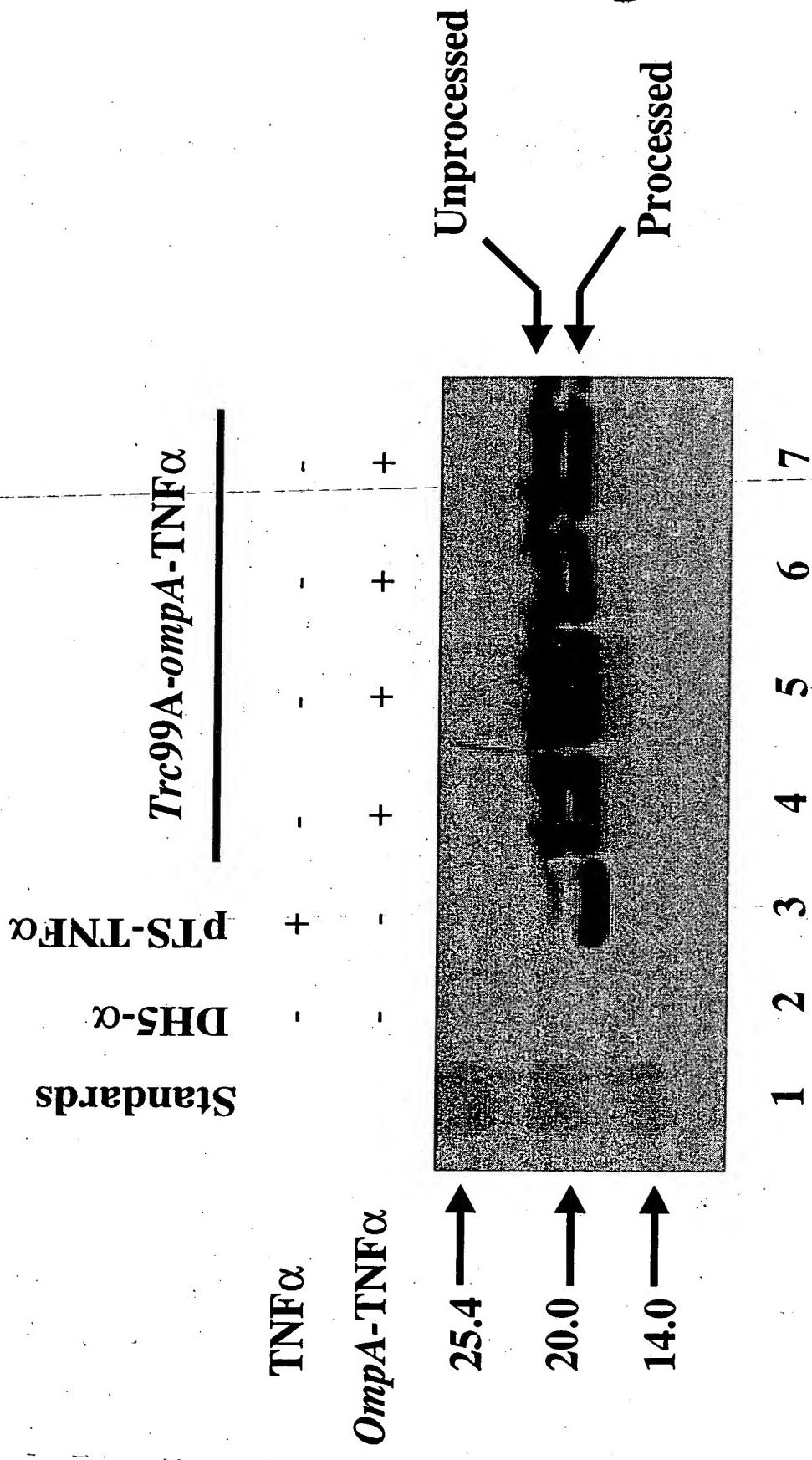
# Expression and processing of a *trc* promoter-driven *ompA-TRAIL* fusion gene product in JM109 bacteria.



**FIG. 5**

ATG AAA AAG ACA GCT ATC GCG ATT GCA GTG GCA CTG GCT GGT TTC  
 M K K T A I A I A V A L A G F  
  
 GCT ACC GTA GCG CAG GCC CAT ATG GCT AAC GAG CTG AAG CAG ATG  
 A T V A Q A H M A N E L K Q M  
  
 CAG GAC AAG TAC TCC AAA AGT GGC ATT GCT TGT TTC TTA AAA GAA  
 Q D K Y S K S G I A C F L K E  
  
 GAT GAC AGT TAT TGG GAC CCC AAT GAC GAA GAG AGT ATG AAC AGC  
 D D S Y W D P N D E E S M N S  
  
 CCC TGC TGG CAA GTC AAG TGG CAA CTC CGT CAG CTC GTT AGA AAG  
 P C W Q V K W Q L R Q L V R K  
  
 ATG ATT TTG AGA ACC TCT GAG GAA ACC ATT TCT ACA GTT CAA GAA  
 M I L R T S E E T I S T V Q E  
  
 AAG CAA CAA AAT ATT TCT CCC CTA GTG AGA GAA AGA GGT CCT CAG  
 K Q Q N I S P L V R E R G P Q  
  
 AGA GTA GCA GCT CAC ATA ACT GGG ACC AGA GGA AGA AGC AAC ACA  
 R V A A H I T G T R G R S N T  
  
 TTG TCT TCT CCA AAC TCC AAG AAT GAA AAG GCT CTG GGC CGC AAA  
 L S S P N S K N E K A L G R K  
  
 ATA AAC TCC TGG GAA TCA TCA AGG AGT GGG CAT TCA TTC CTG AGC  
 I N S W E S S R S G H S F L S  
  
 AAC TTG CAC TTG AGG AAT GGT GAA CTG GTC ATC CAT GAA AAA GGG  
 N L H L R N G E L V I H E K G  
  
 TTT TAC TAC ATC TAT TCC CAA ACA TAC TTT CGA TTT CAG GAG GAA  
 F Y Y I Y S Q T Y F R F Q E E  
  
 ATA AAA GAA AAC ACA AAG AAC GAC AAA CAA ATG GTC CAA TAT ATT  
 I K E N T K N D K Q M V Q Y I  
  
 TAC AAA TAC ACA AGT TAT CCT GAC CCT ATA TTG TTG ATG AAA AGT  
 Y K Y T S Y P D P I L L M K S  
  
 GCT AGA AAT AGT TGT TGG TCT AAA GAT GCA GAA TAT GGA CTC TAT  
 A R N S C W S K D A E Y G L Y  
  
 TCC ATC TAT CAA GGG GGA ATA TTT GAG CTT AAG GAA AAT GAC AGA  
 S I Y Q G G I F E L K E N D R  
  
 ATT TTT GTT TCT GTA ACA AAT GAG CAC TTG ATA GAC ATG GAC CAT  
 I F V S V T N E H L I D M D H  
  
 GAA GCC AGT TTT TTC GGG GCC TTT TTA GTT GGC TAA  
 E A S F F G A F L V G \*

**Expression and processing of a *trc* promoter-driven *ompA-TNF $\alpha$*  fusion gene product in JM109 bacteria.**

**FIG. 7**

ATG AAA AAG ACG GCT CTG GCG CTT CTG CTC TTG CTG TTA GCG CTG  
 M K K T A L A L L L L L A L  
 ACT AGT GTA GCG CAG GCC GCT CCT ACT AGC TCG AGC ACT AAG AAA  
 T S V A Q A A P T S S S T K K  
 ACT CAA CTG CAA TTG GAG CAT CTG CTG GAT CTG CAG ATG ATT  
 T Q L Q L E H L L D L Q M I  
 CTG AAT GGC ATC AAT AAC TAC AAG AAC CCT AAG CTG ACT CGC ATG  
 L N G I N N Y K N P K L T R M  
 CTG ACT TTC AAA TTC TAC ATG CCG AAA AAG GCT ACC GAG CTC AAA  
 L T F K F Y M P K K A T E L K  
 CAT CTC CAG TGC CTG GAA GAG GAA CTG AAG CCG CTG GAG GAA GTA  
 H L Q C L E E E L K P L E E V  
 CTT AAC CTG GCA CAG TCT AAG AAC TTC CAC CTG CGT CCG CGT GAC  
 L N L A Q S K N F H L R P R D  
 CTG ATC TCC AAC ATC AAT GTA ATC GTT CTT GAG CTG AAG GGA TCC  
 L I S N I N V I V L E L K G S  
 GAA ACC ACC TTC ATG TGC GAA TAC GCT GAC GAA ACC GCC ACC ATT  
 E T T F M C E Y A D E T A T I  
 GTG GAG TTC CTG AAC CGT TGG ATC ACC TTT GCC CAA TCG ATC ATT  
 V E F L N R W I T F A Q S I I  
 AGC ACG TTA ACT TAA  
 S T L T \*

Periplasmic localization and processing of *ompA*-IL2 fusion proteins.

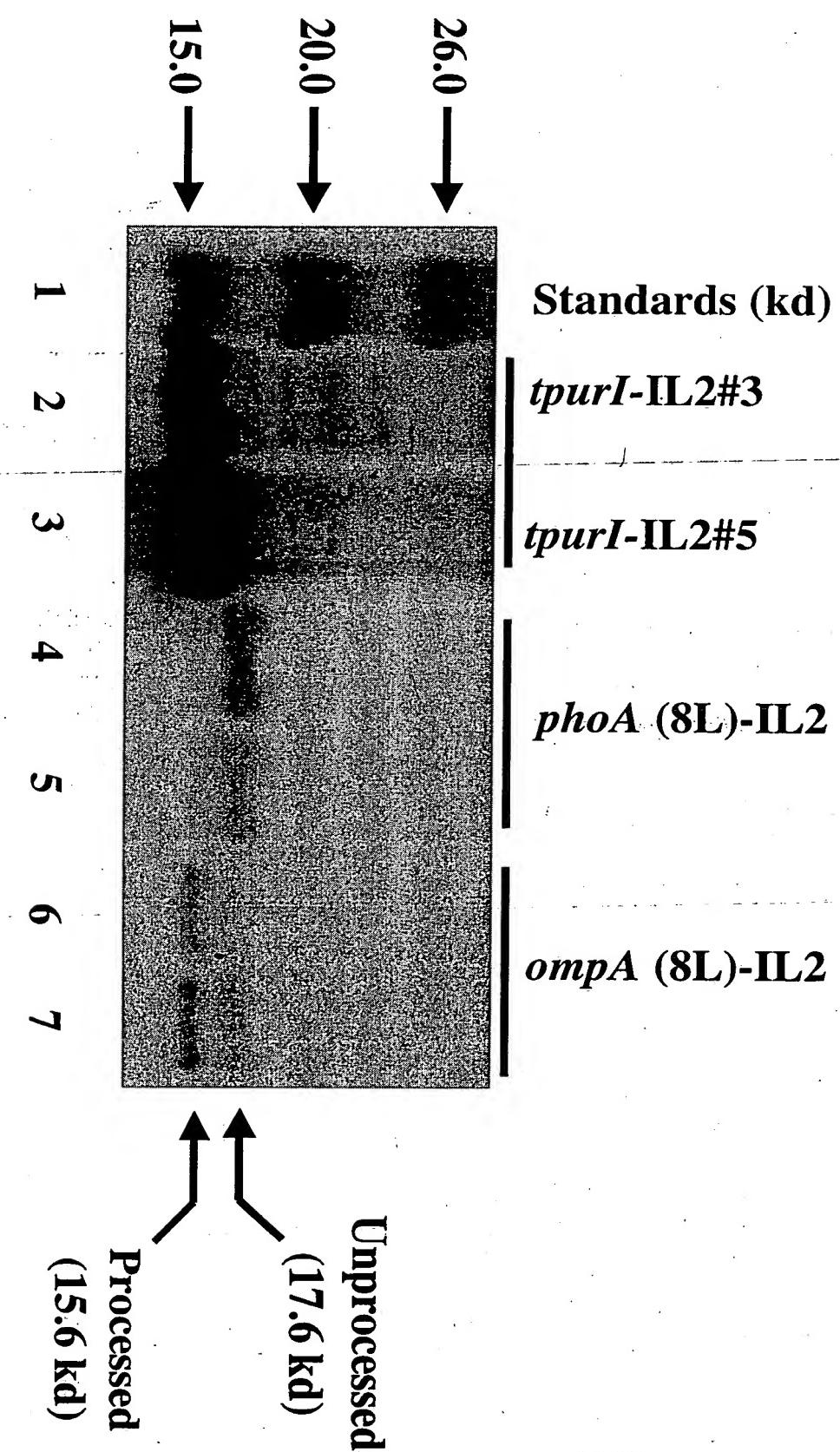


FIG. 9

MB85P131

ATG AAA CAG TCG ACT CTG GCG CTT CTG CTC TTG CTG TTA GCG CTG  
 M K Q S T L A L L L L L A L  
 ACT AGT GTG GCC AAA GCG GCT CCT ACT AGC TCG AGC ACT AAG AAA  
 T S V A K A A P T S S S T K K  
 ACT CAA CTG CAA TTG GAG CAT CTG CTG GAT CTG CAG ATG ATT  
 T Q L Q L E H L L L D L Q M I  
 CTG AAT GGC ATC AAT AAC TAC AAG AAC CCT AAG CTG ACT CGC ATG  
 L N G I N N Y K N P K L T R M  
 CTG ACT TTC AAA TTC TAC ATG CCG AAA AAG GCT ACC GAG CTC AAA  
 L T F K F Y M P K K A T E L K  
 CAT CTC CAG TGC CTG GAA GAG GAA CTG AAG CCG CTG GAG GAA GTA  
 H L Q C L E E E L K P L E E V  
 CTT AAC CTG GCA CAG TCT AAG AAC TTC CAC CTG CGT CCG CGT GAC  
 L N L A Q S K N F H L R P R D  
 CTG ATC TCC AAC ATC AAT GTA ATC GTT CTT GAG CTG AAG GGA TCC  
 L I S N I N V I V L E L K G S  
 GAA ACC ACC TTC ATG TGC GAA TAC GCT GAC GAA ACC GCC ACC ATT  
 E T T F M C E Y A D E T A T I  
 GTG GAG TTC CTG AAC CGT TGG ATC ACC TTT GCC CAA TCG ATC ATT  
 V E F L N R W I T F A Q S I I  
 AGC ACG TTA ACT TAA  
 S T L T \*

FIG. 10

Antitumor efficacy of pTS-BrpTNF $\alpha$  Clone 2 in a staged  
Colon 38 tumor model.

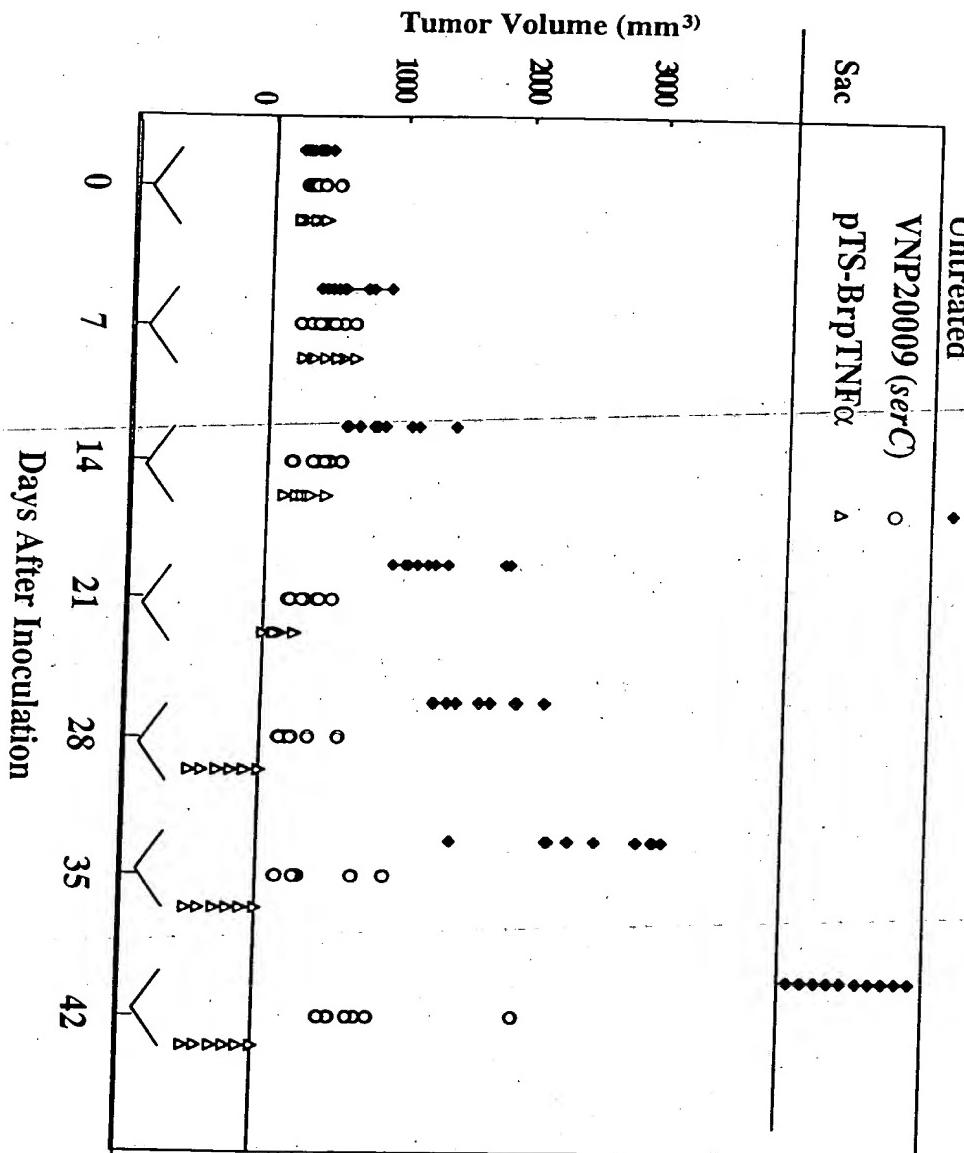


FIG. 11

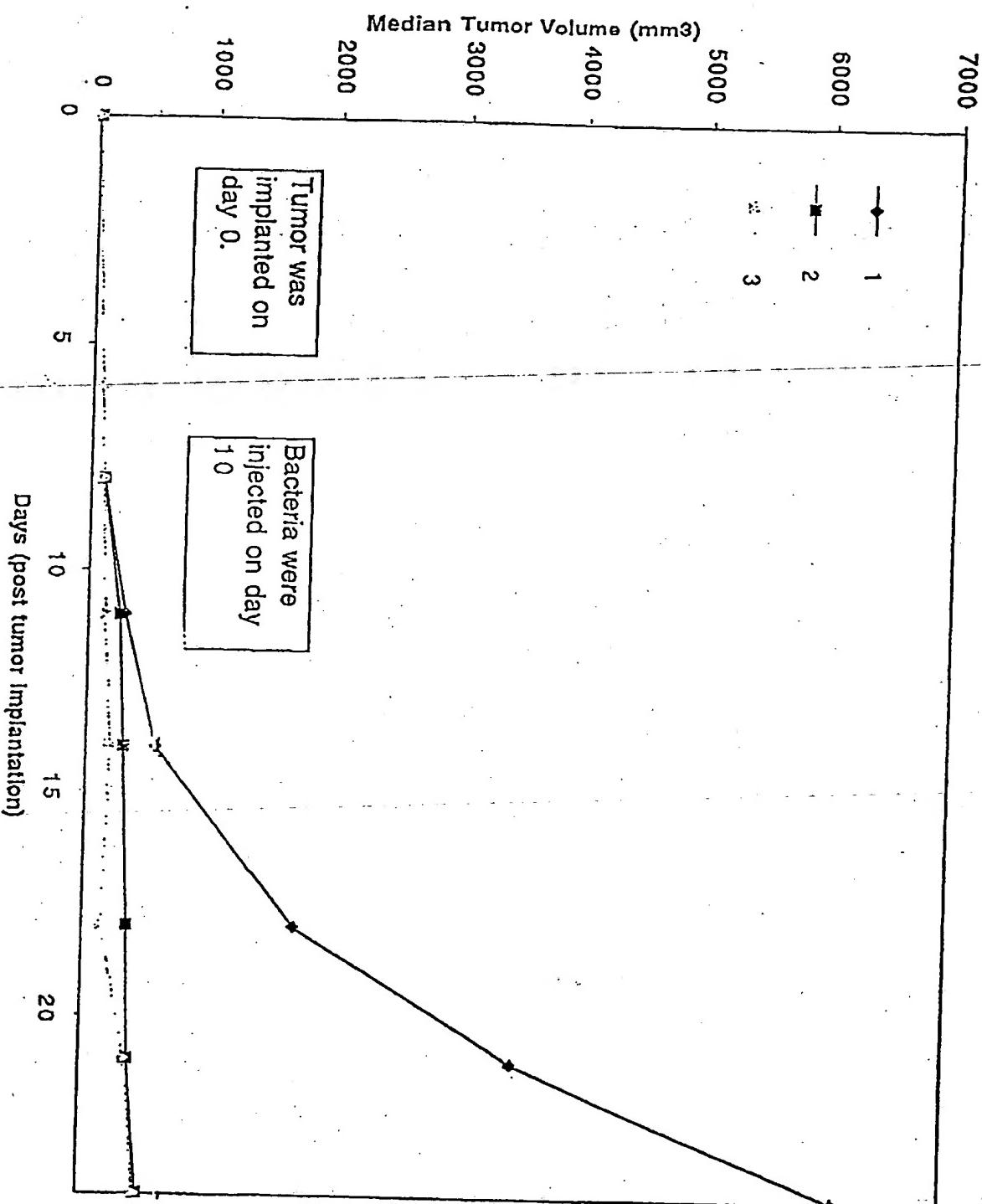


FIG. 12

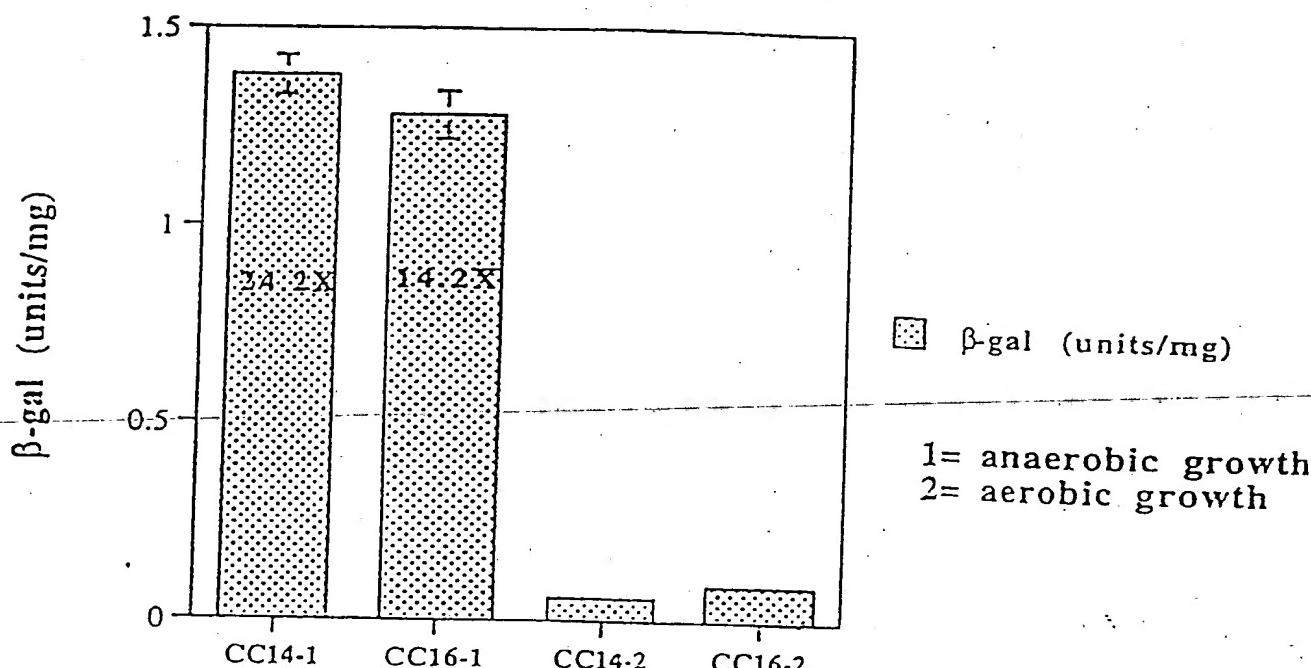
$\beta$ -gal activity in strains carrying pepT $\beta$ gal

FIG. 13A

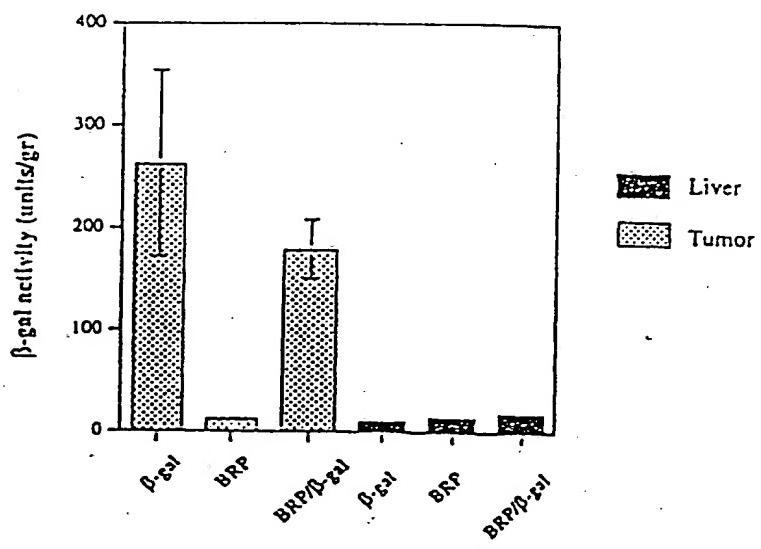
 $\beta$ -gal activity *in vivo*, pepT $\beta$ -gal  $\pm$  BRP

FIG. 13B

Expression of  $\beta$ -gal using  
TET promoter

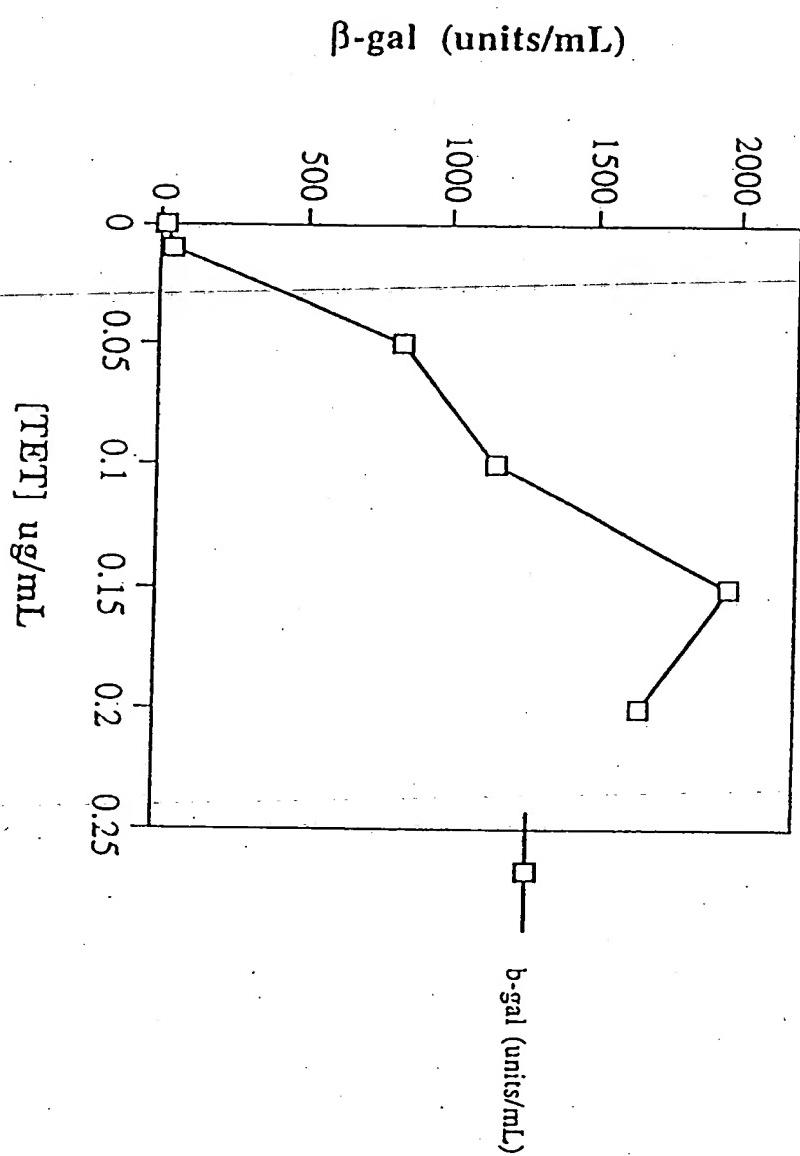
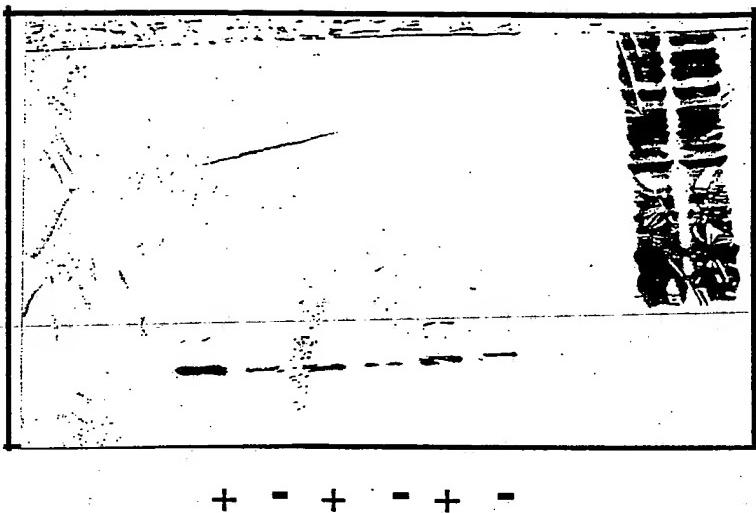


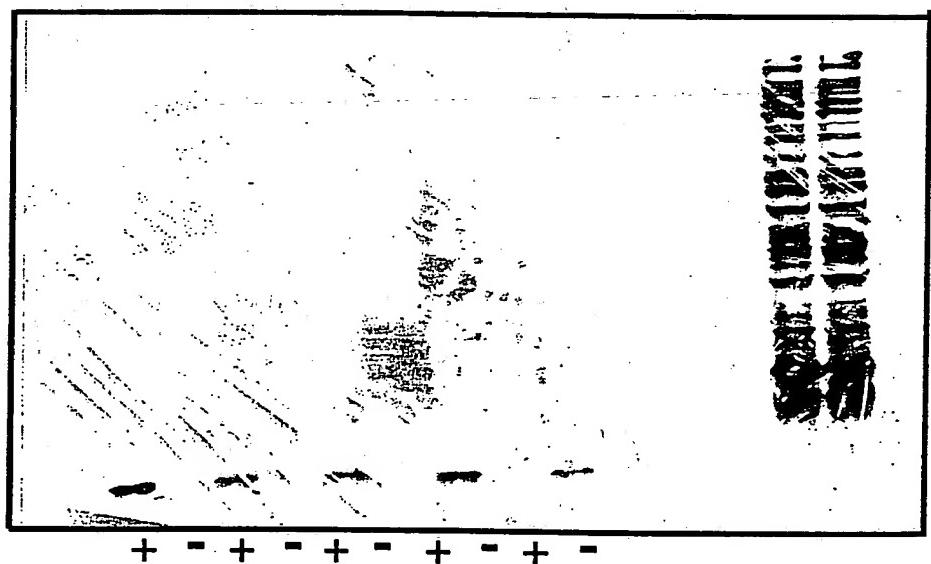
FIG. 14

**A.**

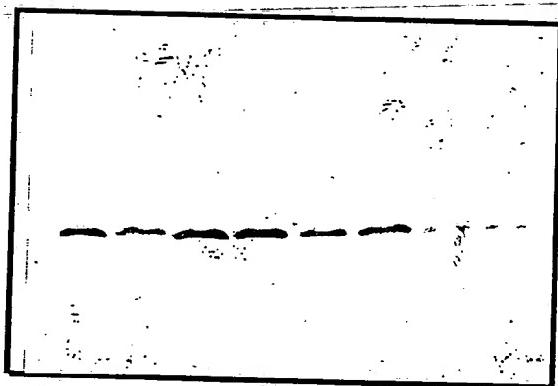
~25 kD →  
HexaHIS-endostatin

**B.**

~25 kD →  
HexaHIS-endostatin

**FIG. 15**

**~25kD** →  
**HexaHIS-endostatin**



**FIG. 16**

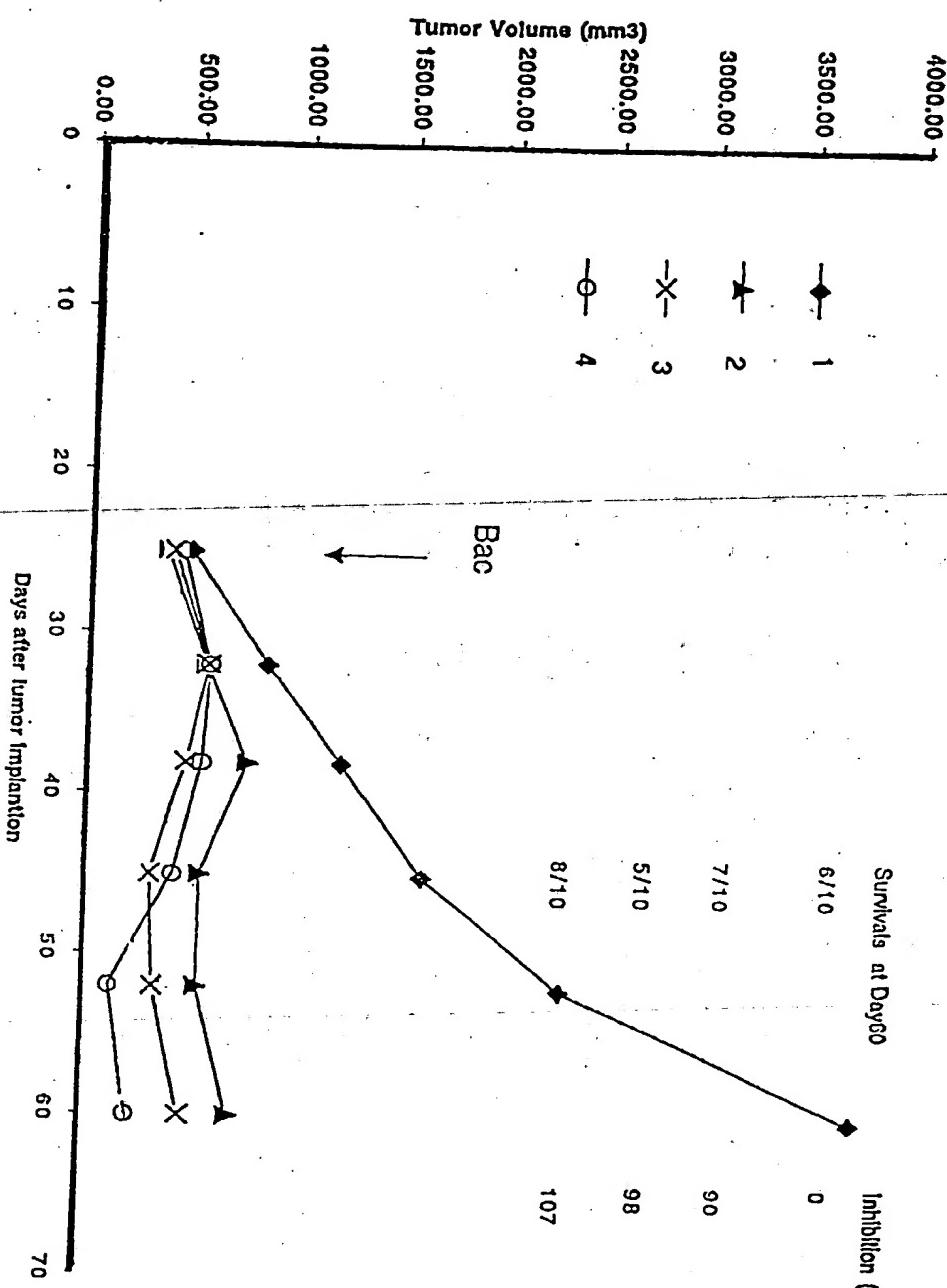
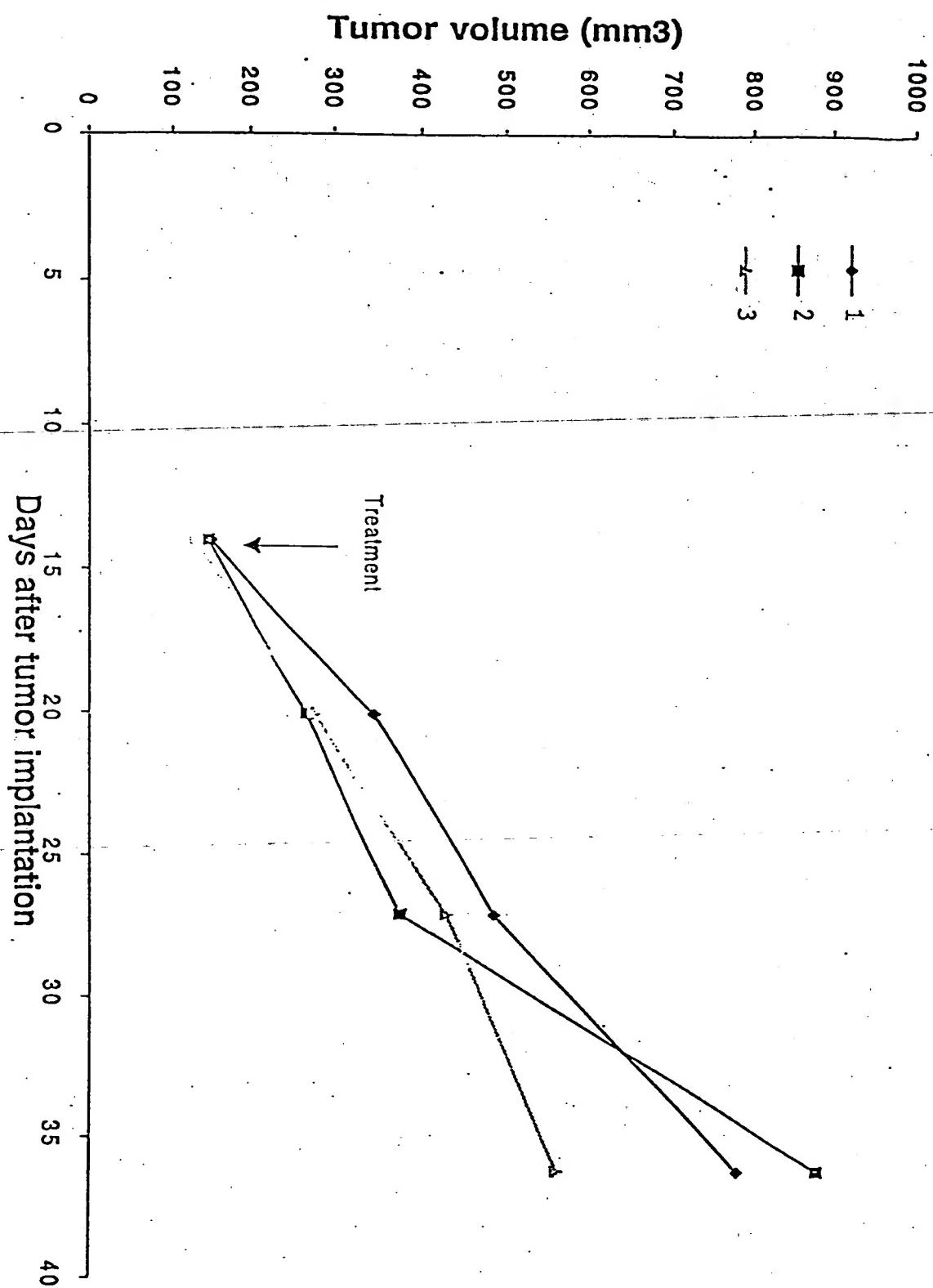
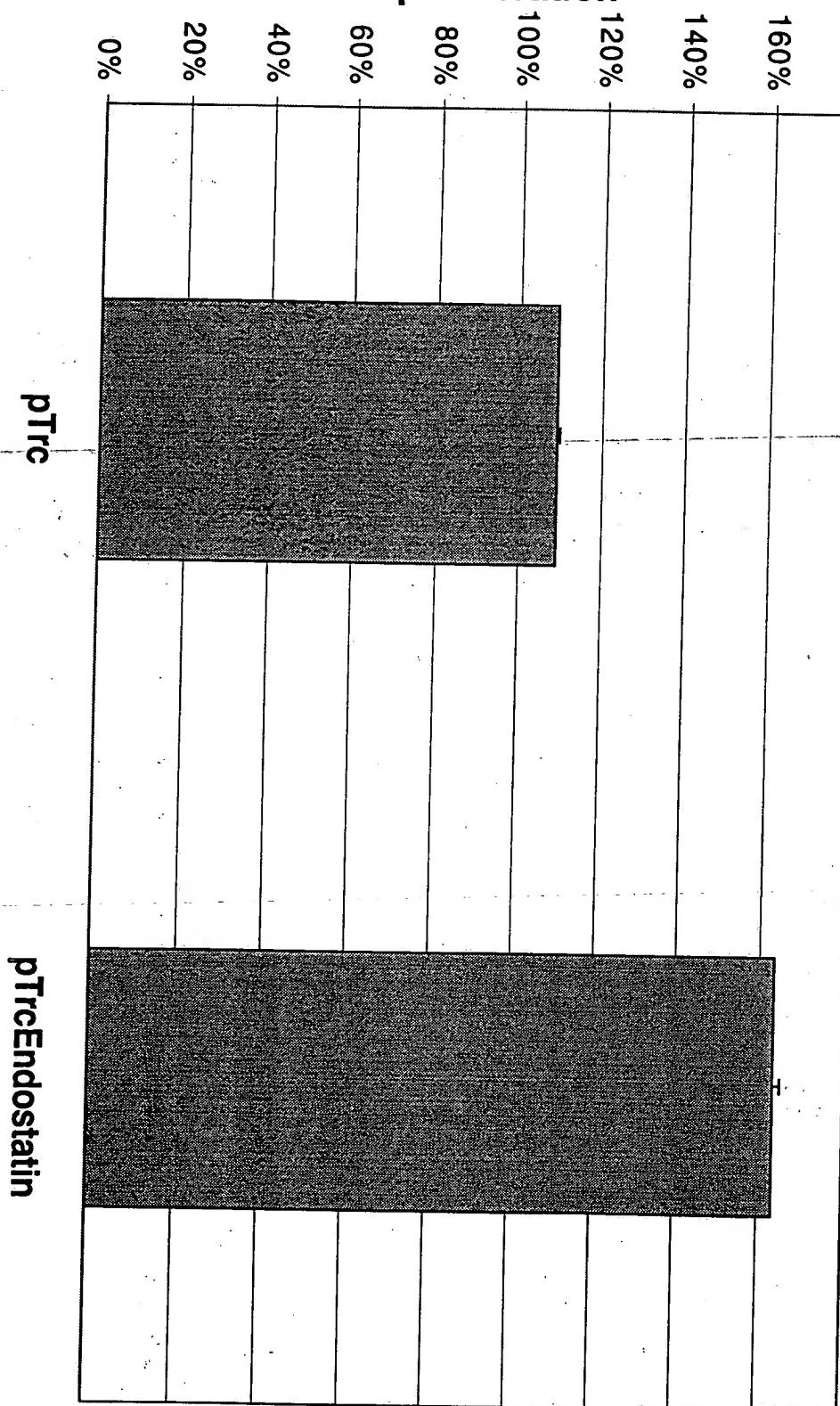


FIG. 17

**FIG. 18**

### Inhibition of endothelial cell proliferation



**Inhibitory activity of lysates from a *Salmonella* strain expressing human endostatin**

**FIG. 19**

### Inhibition of endothelial cell proliferation

### Inhibitory activity of lysates from *Salmonella* strains expressing anti-angiogenic peptides

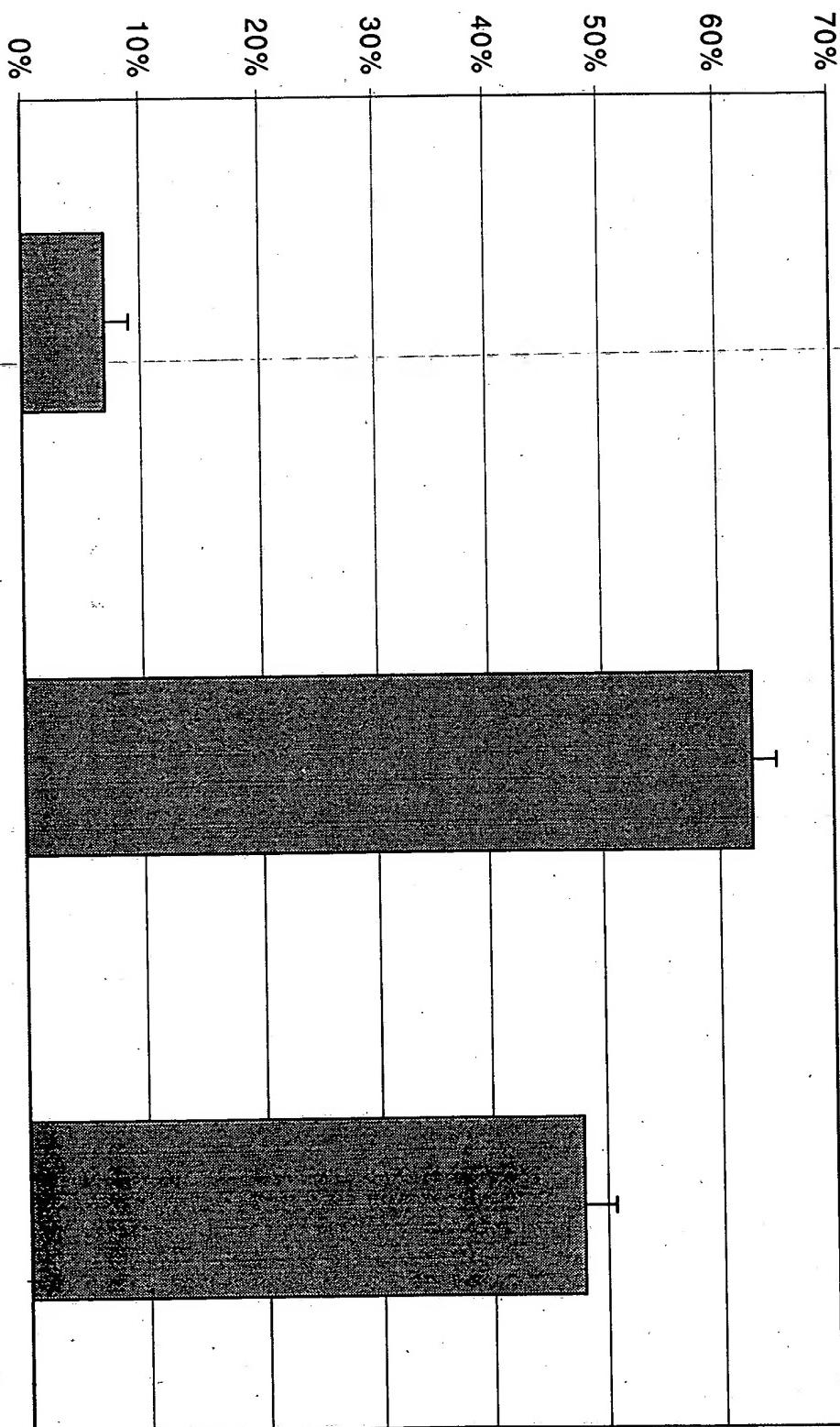
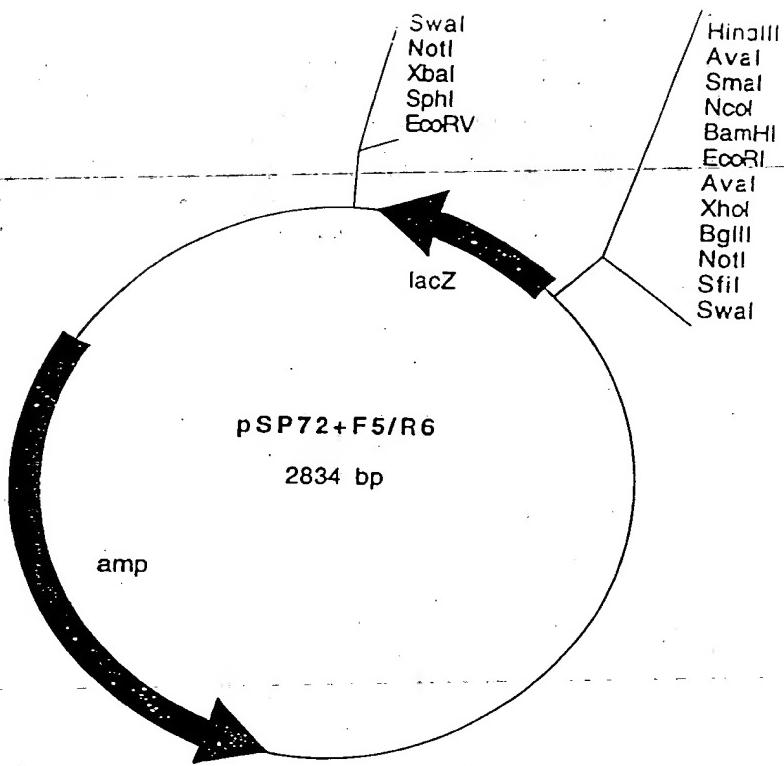


FIG. 20



**FIG. 21**

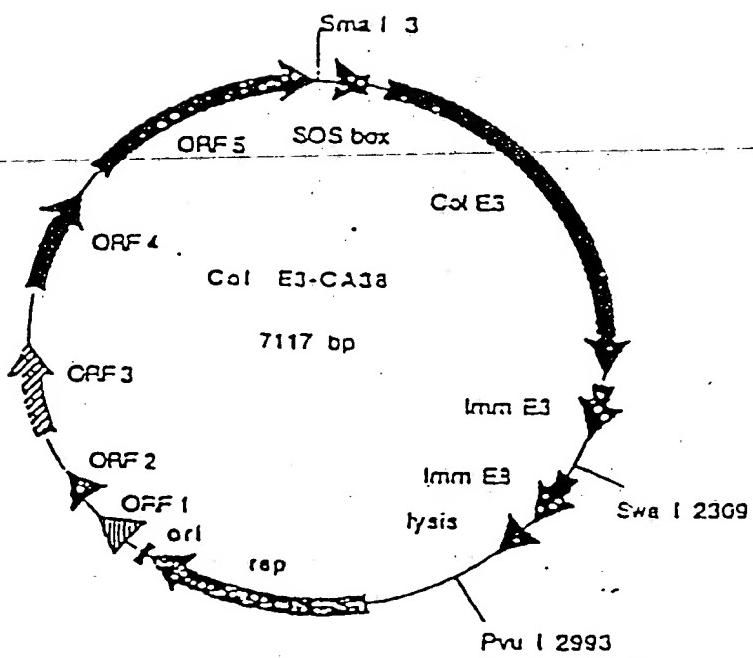


FIG. 22

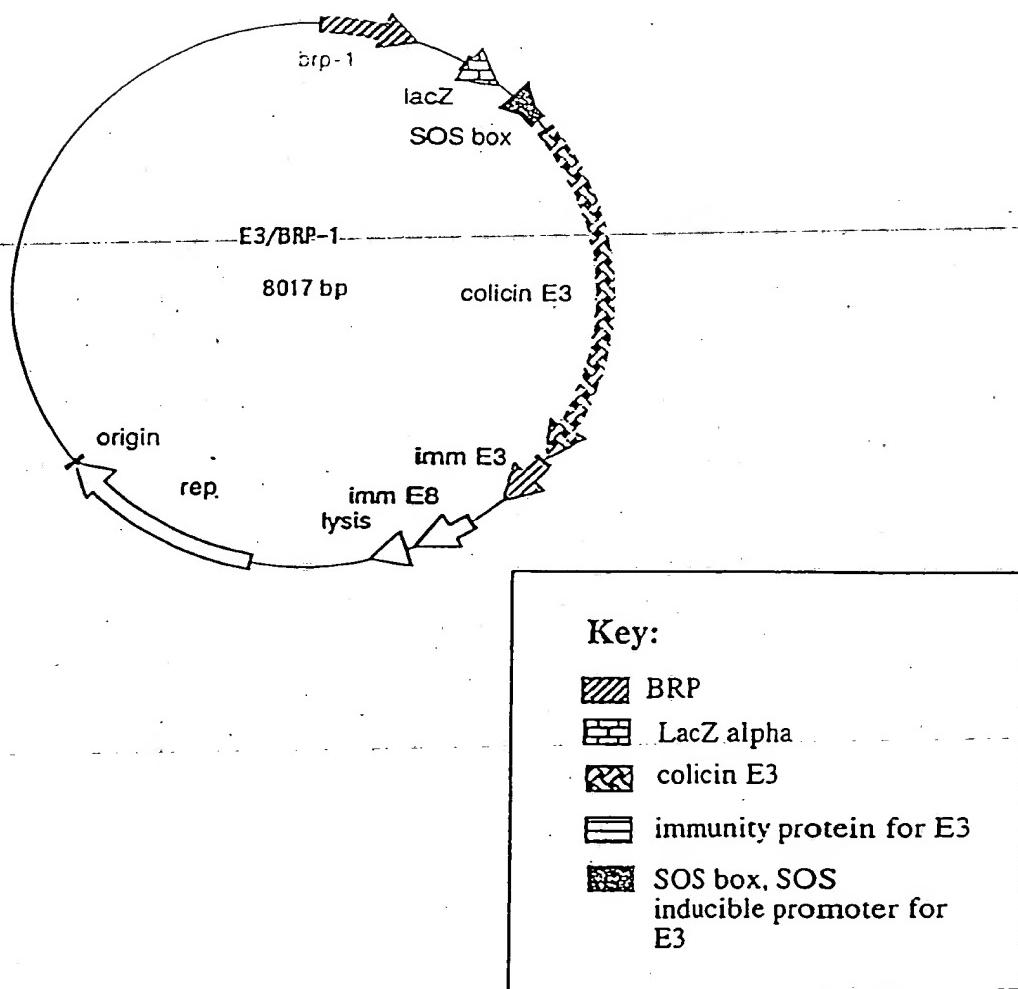
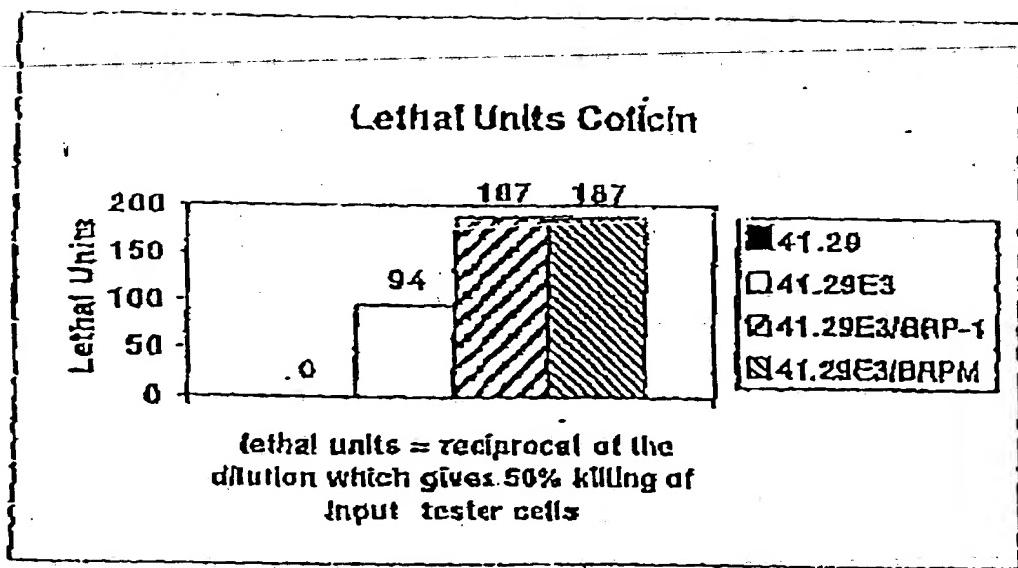


FIG. 23



**FIG. 24**

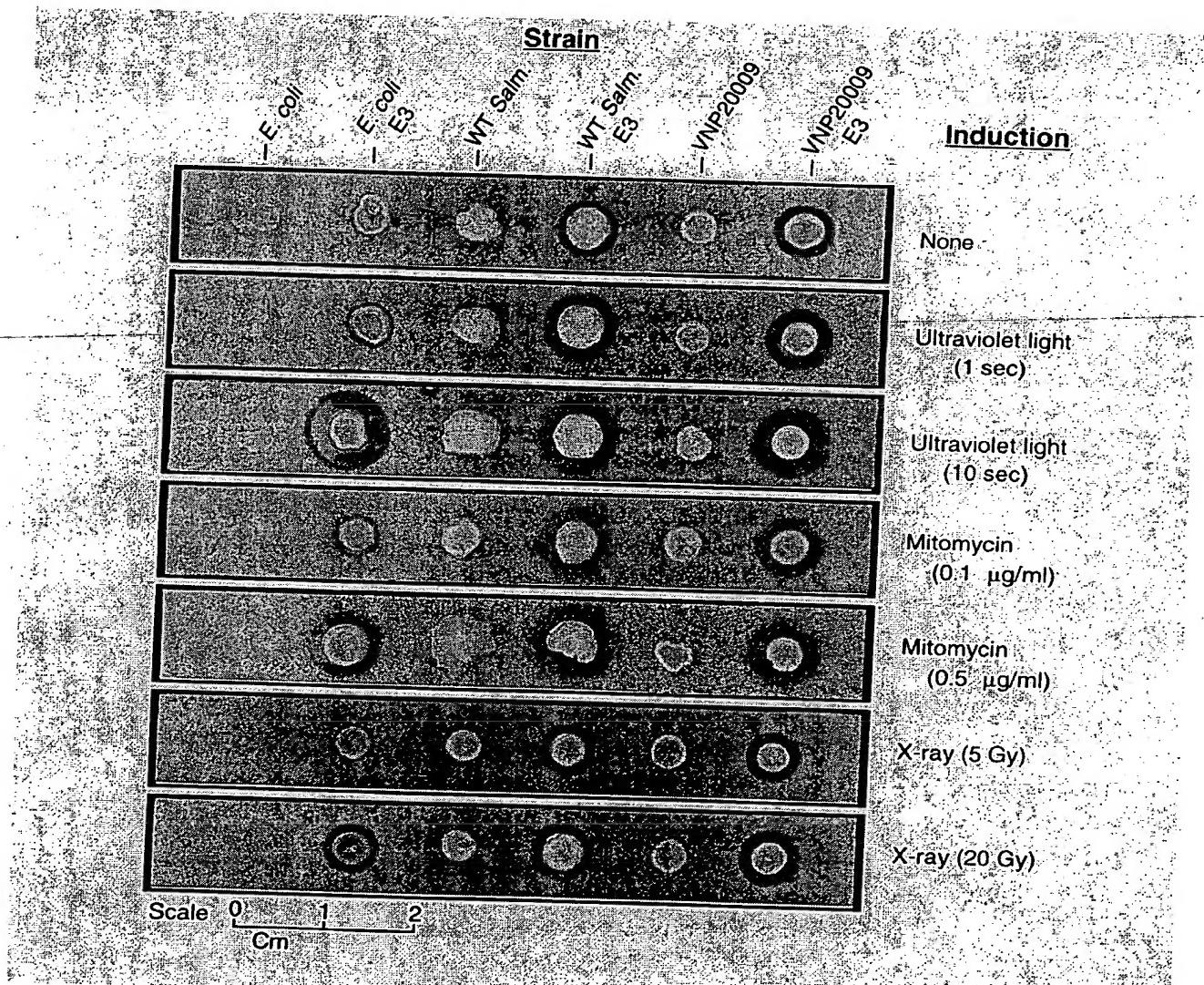


FIG. 25

## Efficacy of 41.2.9/ColE3 on C38 Murine Colon Carcinoma

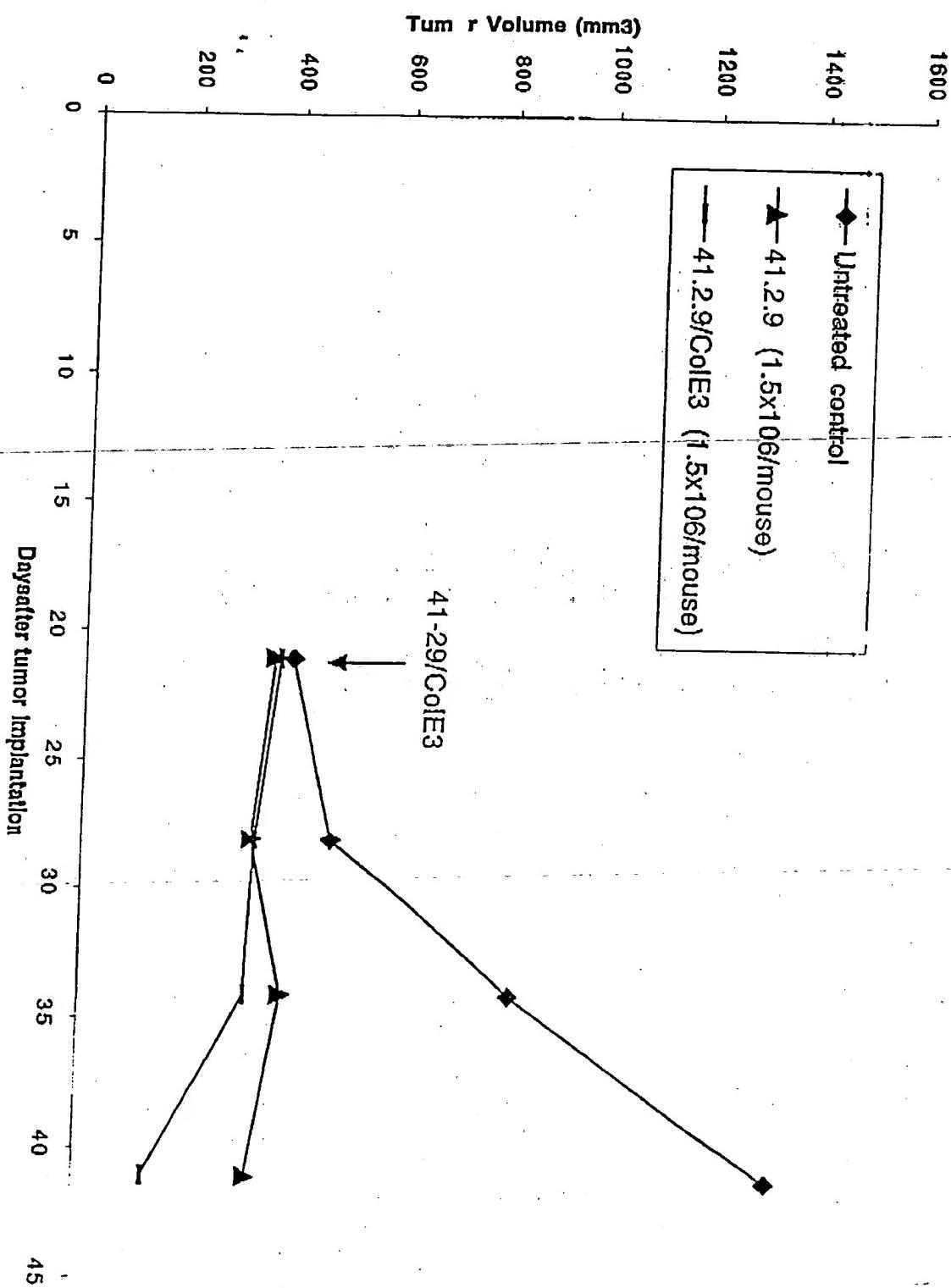
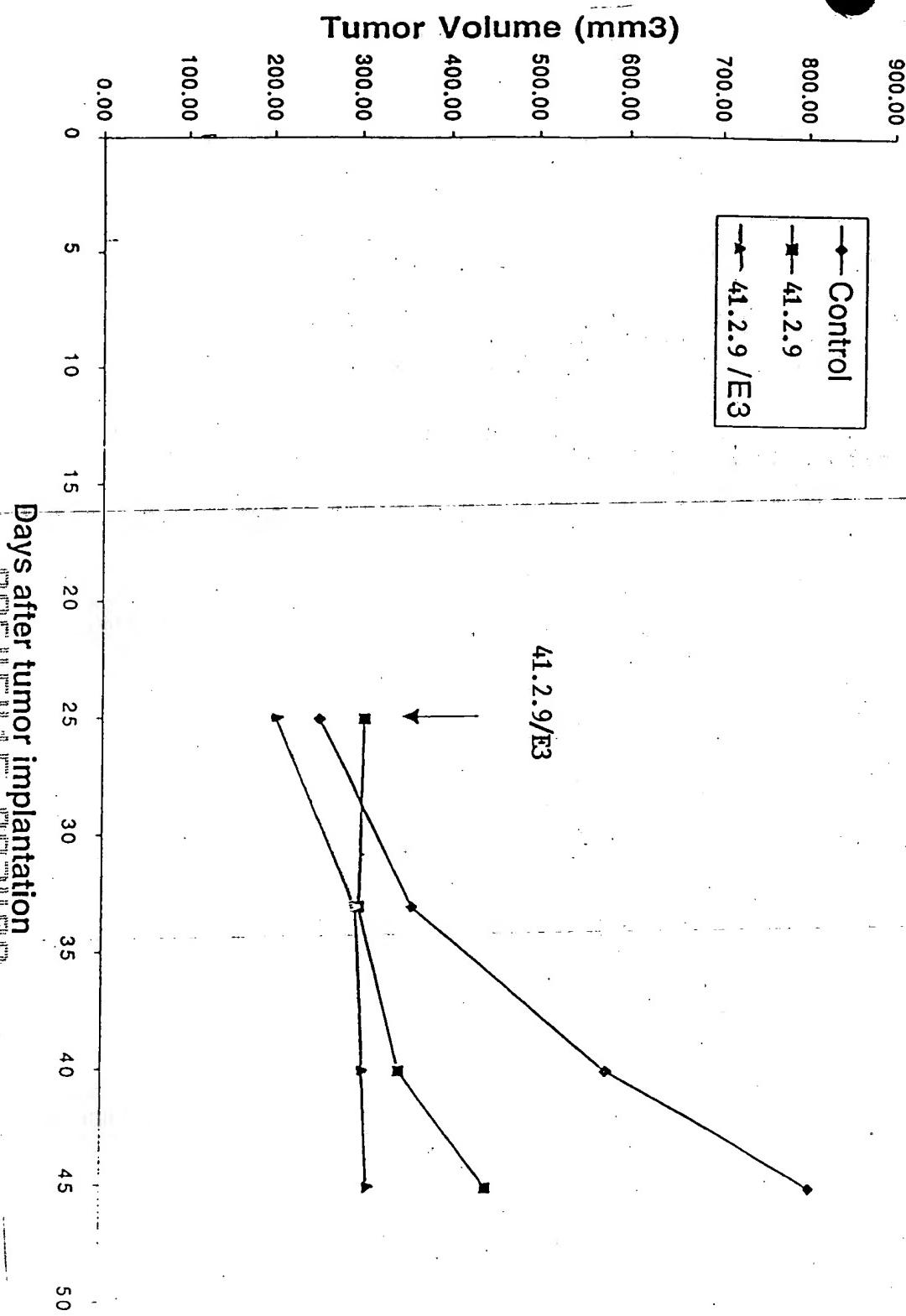


FIG. 26

Anti-tumor Activity of 41.2.9 /Col/E3 on DLD1 Human Colon Carcinoma in  
Nu/Nu Mice



**FIG. 27**

8002-059

(SHEET 28 OF 41)

Efficacy of 41.2.9/ColE3 on B16 Murine Melanoma

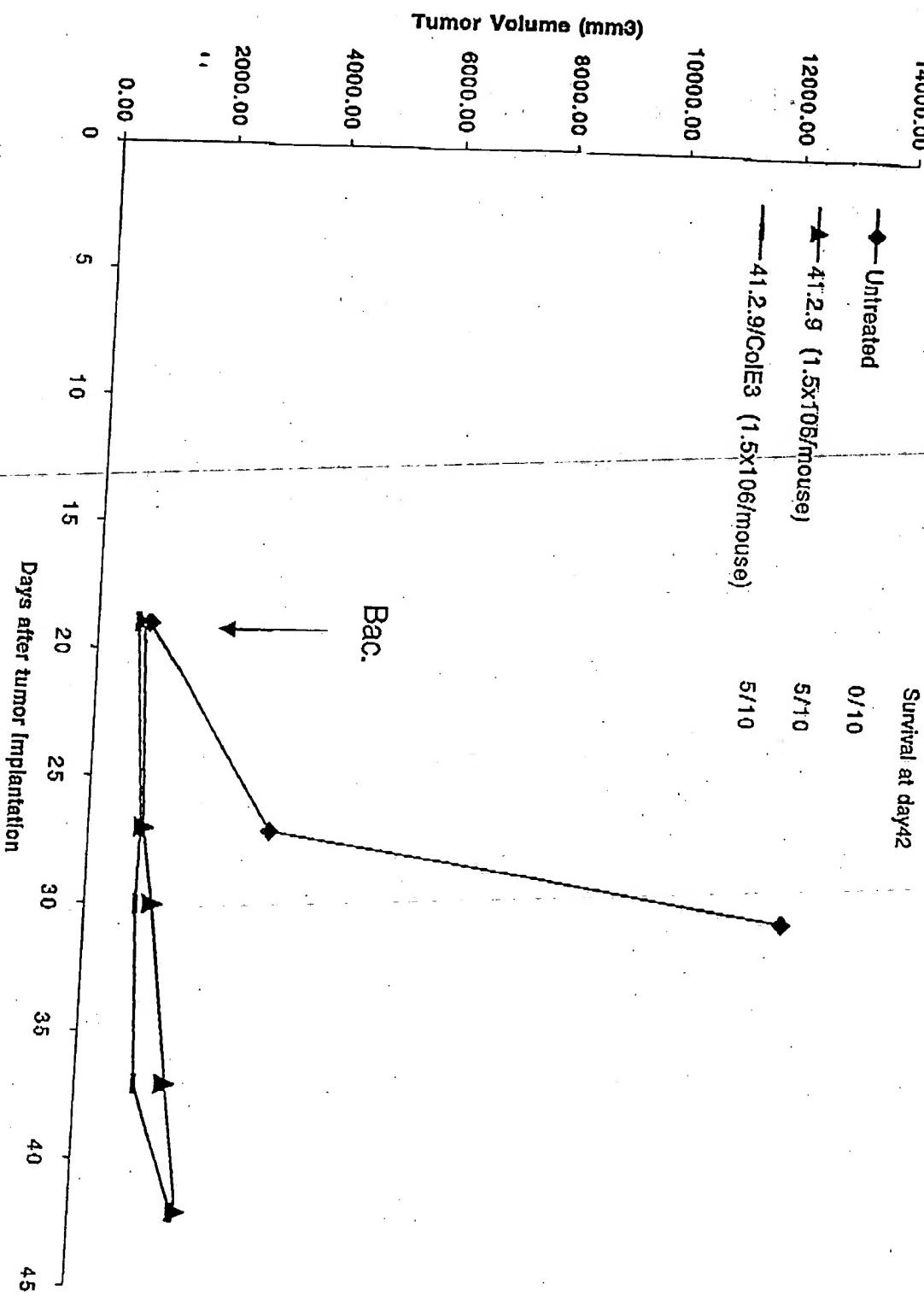
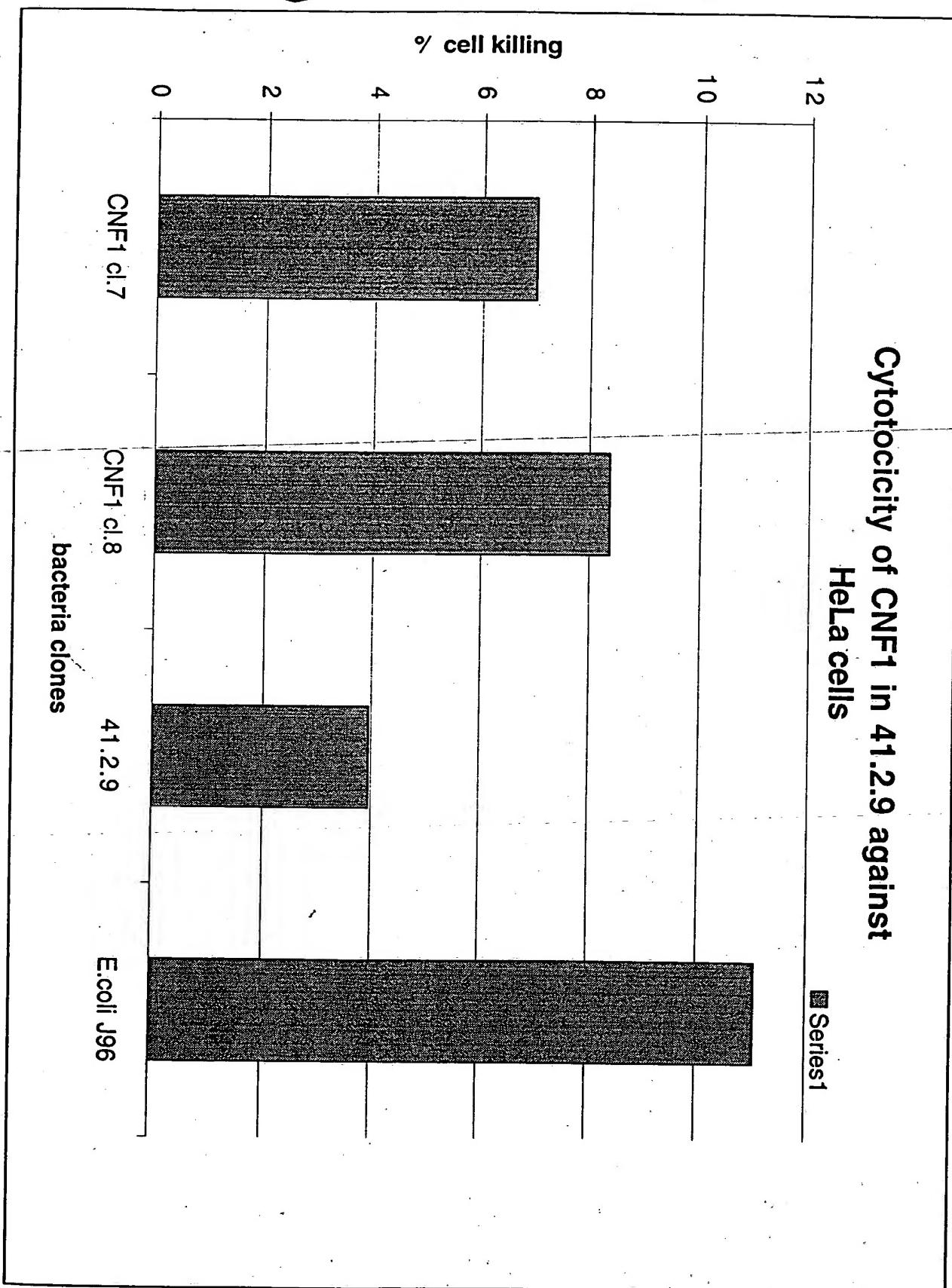


FIG. 28



**FIG. 29**

8002-059

(SHEET) 30 OF 41

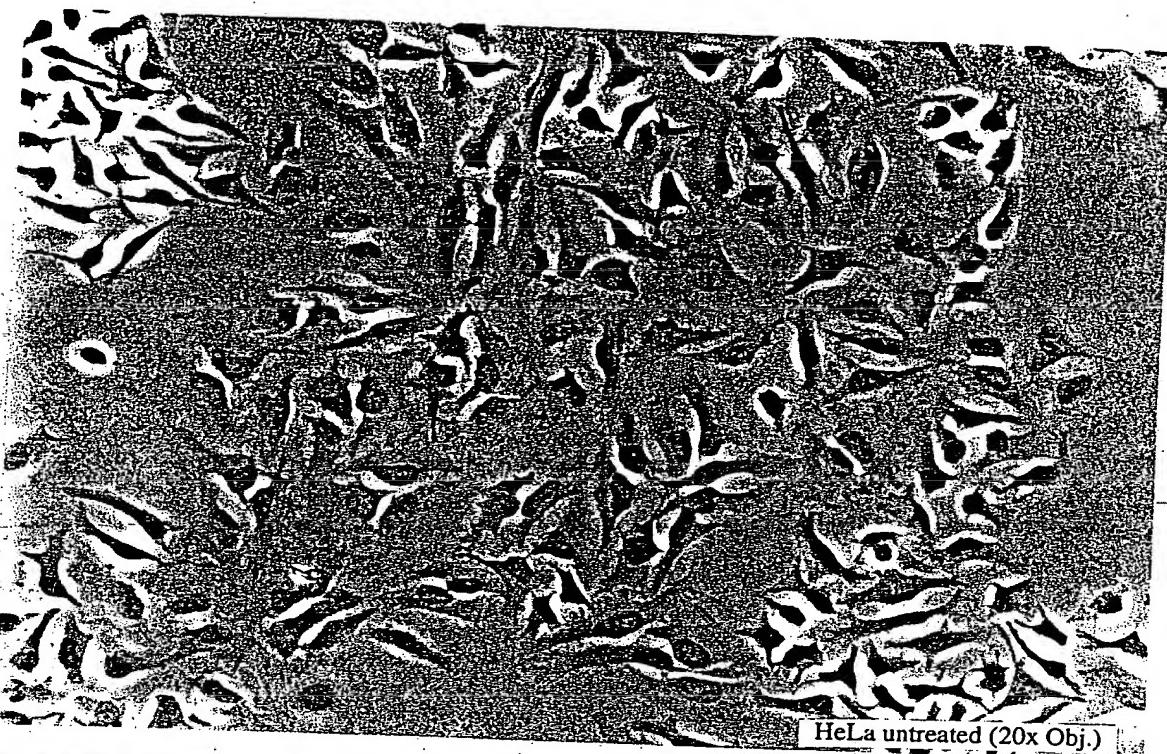
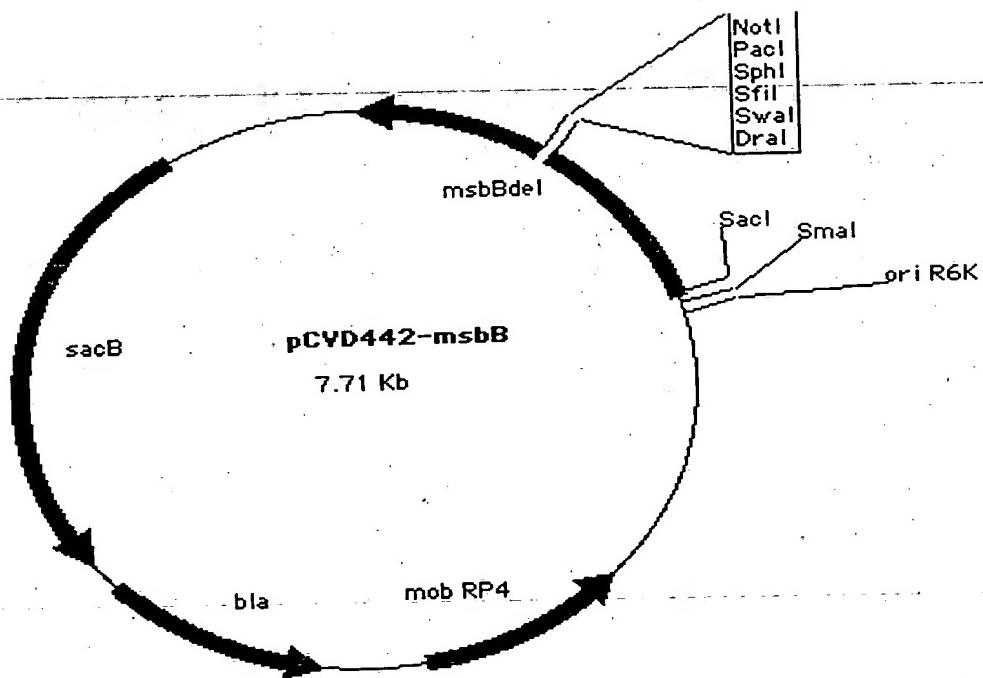


FIG. 30

GATATCATTC TGGCCTCTGA CGTTGTGATG GTCGCCACGTG CCGATCTGGG CGTTGAAATC GGCGATCCGG	70
AGCTGGTTGG TATCCAGAAA GCGCTGATTC GCCGTGCGCG TCAGCTAAC CGCCGAGTCA TCACCGCAAC	140
GCAAATGATG GAGTCGATGA TCACCAACCC GATGCCGACC CGTGCGGAAG TGATGGACGT GGCGAACGCC	210
GTCCCTGGATG GCACGGATGC GGTTATGCTG TCTGCCGAAA CGCGAGCCGG TCAGTATCCT TCTGAAACCG	280
TTGCCGCAAT GGGCGCGCGTC TGCCCTGGCG CAGAAAAAAT CCCCAGCATC AATGTGTCTA AACACCGTCT	350
CGACGTGCAG TTGACAACG TTGAAGAACG CATTGCCATG TCTGCGATGT ATGCGGAAA CCATCTGAAA	420
GGCGTTACCG CGATCATCAC CATGACGGAA TCCGGTGTAA CGCGCCTAAT GACTTCCCGT ATCAGCTCCG	490
GCCTGCCGAT TTTCGCCATG TCGCGCCATG AACGCACGCT GAACCTGACC GCGCCTATC GCGGAGTAAC	560
GCCGGTGCAT TTGATAGCG CGGCTGATGG CGTGTGCGCG CCACATGAAG CTGTTAATCT CCTGCCGCGAT	630
AAAGGGTATC TGGTTTCCGG CGACCTGGTT ATCGTGACCC AGGGCGATGT CATGAGCACC GTCGGTTCAA	700
CCAATACAC GCGGCCGCC CTTTAATTAA CCCCCCATGC GGGGGGCCAT ATAGGCCGGG GATTTAAATG	770
CAAACGTCCG CCGAAACGCC GACGCACTGT GTTCCAGATA TAGTCAAAAA CGGAAATTACC CTGATTATGA	840
AACATGCCG CCATTTTTG CCCCCTGAGAG GCCATCAGCA TGGCTGGAAT GTCGACGGCC CAGCCATGCC	910
GTACGAGAAA AATGACTTT TCGTCGTAC GACGCATCTC CTCGATAATC TCCAGACCTT CCCAGTCAC	980
ACGCTGTTGA ATTTCGCG GACCGCGCAT CGCCAACCTCA GCCATCATCG CCATTGCCCTG TGGCCGGTG	1050
GCGAACATCT CATCGACAAT CGCTTCGCGC TCAGCTTCGC TACGCTGCGG AAAGCACAAC GACAGATTAA	1120
TTAGGCCCG GCGACGAGAA CTCTTCCCCA GCCGTCGGC AAAACGCCCG AGCGTCGCCA GCAAAGGGTC	1190
GCGGAATGAT GCCGGTGTATA ATGCGATCCC CGCCATTGCC GCGCGGCCA ACCAGGGCC CCAATACTGT	1260
GGATAGCGAA AGGATTTTC GAATTCAAGGG ATATACTCAC TATTATTTT TTGGTTTCC ATGCTTTCC	1330
AGGGTCTGCT GACCGAAGAA GGAATTGTGA ATAGTGTAGC GACGTCTGCG TCTCACACAA AACAAAAAAG	1400
CCGGCACACA TCGCGTACCG GCTCTGTCAG CGCATTGTT AATCGAAGCG CAGTTGCCGC AGAACCTCTT	1470
TCACCTGTGC CAGGTATTCA CGACGATCTG ACCCCGTCAG ACCTTCGTG CGCGGCAATT TTGCTGTCAG	1530
AGGGTTAACG GCTTGCTGGT TGATC	1555

FIG. 31

**FIG. 32**

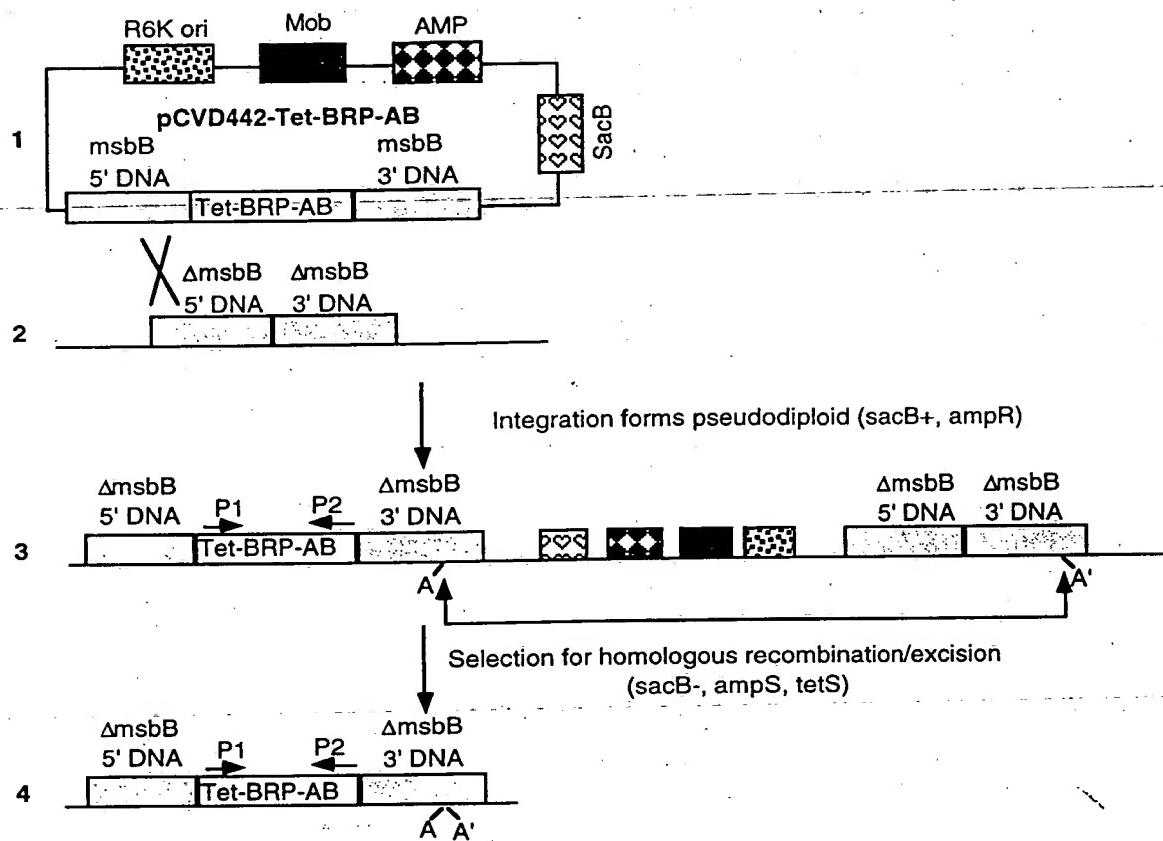


FIG.33

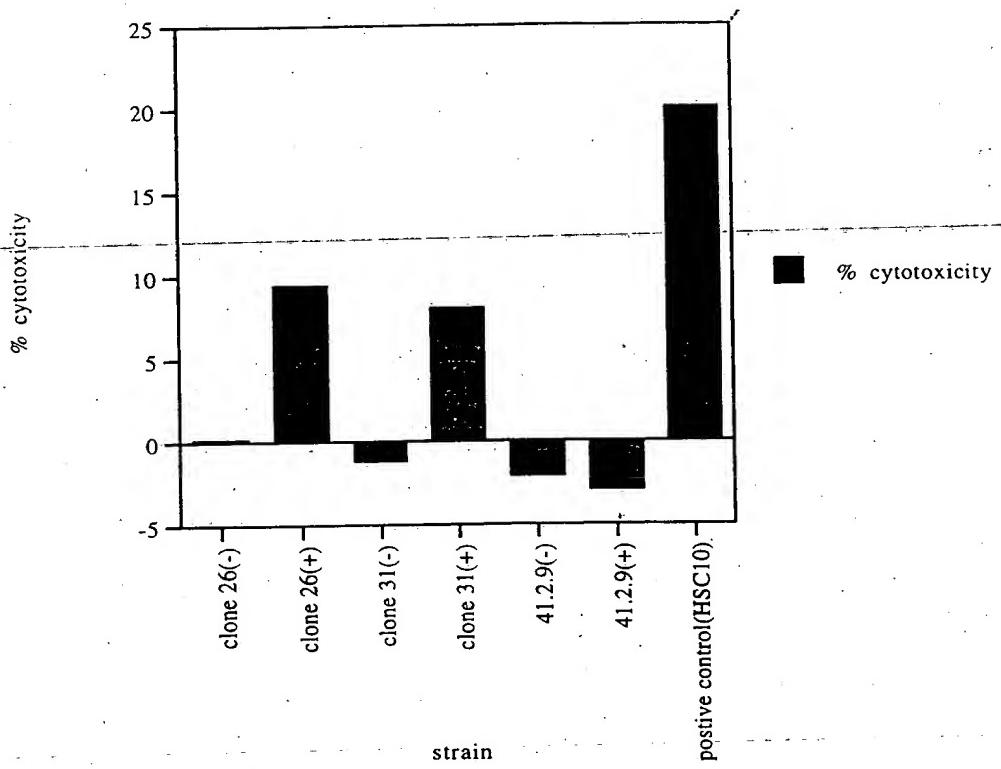
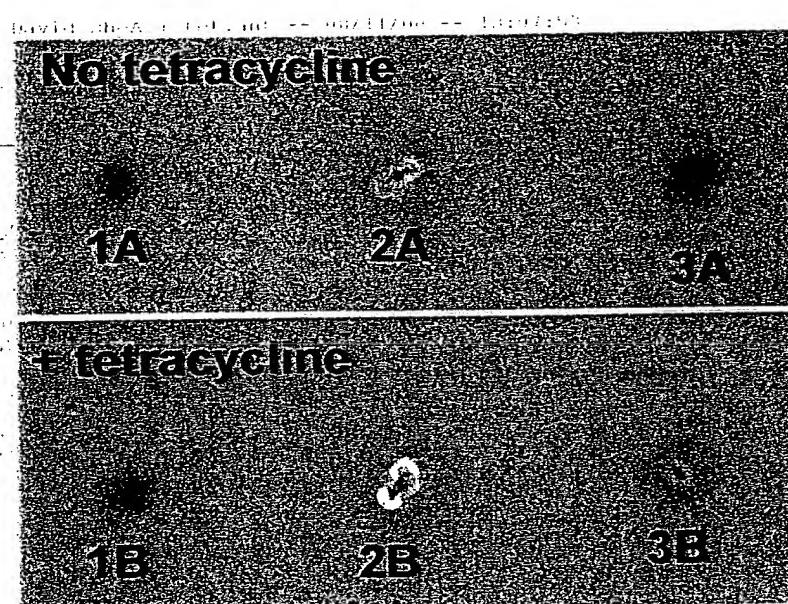


FIG. 34



**FIG. 35**

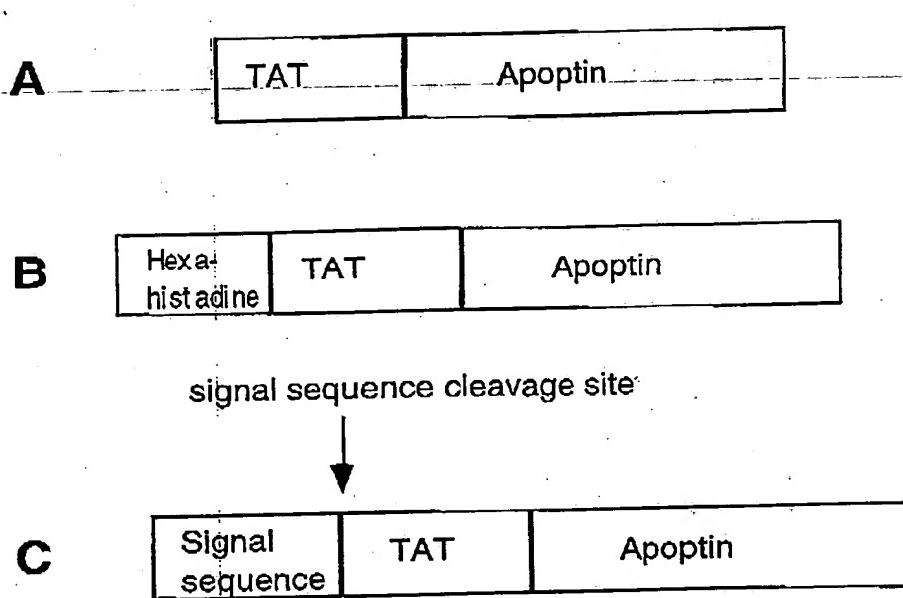


FIG. 36

## Protein Sequence of 616-4 F

page 1

Length of 616-4 F: 551 bp; Listed from: 1 to: 551;  
 Translated from: 7 to: 409 (Entire region);  
 Genetic Code used: Universal; Wed, Aug 16, 2000 1:40 PM

Frame 1

M	A	Y	G	R	K	K	R	R	R	Q	R	R	R	R	M	N			
NAG	ACC	ATG	GCT	TAT	GGC	AGA	AAA	AAA	AGA	AGA	CAG	AGA	AGA	AGA	ATG	AAC			
9											27				36	45			
A	L	Q	E	D	T	P	P	G	P	S	T	V	F	R	P	P	T	S	
GCG	CTG	CAG	GAA	GAT	ACC	CCG	CCG	GGC	CCG	TCC	ACC	GTG	TTT	CGC	CCG	CCG	ACC	TCC	
60												87				96	105		
S	R	P	L	E	T	P	H	C	R	E	I	R	I	G	I	A	G	T	
TCC	CGC	CCG	CTG	GAA	ACG	CCG	CAT	TGC	CGC	GAA	ATC	CGC	ATC	GGC	ATC	GCG	GGC	ATC	
117																153	162		
126																			
T	I	T	L	S	L	C	G	C	A	N	A	R	A	P	T	L	R	S	
ACC	ATC	ACC	CTG	TCC	CTG	TGC	GGC	TGC	GGC	AAC	GCG	CGC	GCG	CGC	CCG	ACC	CTG	CGC	TCC
174																		219	
183																			
A	T	A	D	N	S	E	N	T	G	F	K	N	V	P	D	L	R	T	
GCG	ACC	GCG	GAT	AAC	TCC	GAA	AAC	ACC	GGC	TTT	AAA	AAC	GTC	CCG	GAT	CTG	CGC	ACC	
231																		276	
240																			
D	Q	P	K	P	P	S	K	K	R	S	C	D	P	S	E	Y	R	V	
GAT	CAG	CCG	AAA	CCG	CCG	TCC	AAA	AAA	CGC	TCC	TGC	GAT	CCG	TCC	GAA	TAT	CGC	GTC	
288																		333	
297																			
S	E	L	K	E	S	L	-	I	T	-	T	P	S	R	P	R	T	A	R
TCC	GAA	CTG	AAA	GAA	TCC	CTG	ATC	ACC	ACC	ACC	CCG	TCC	CGC	CCG	CGC	ACC	GCC	CGC	
345																		390	
354																			
R	C	I	R	L	.														
CGC	TGC	ATC	CGC	CTC	TGA	AAG	CTT	GGC	TGT	TTT	GGC	GGA	TGA	GAG	AAG	ATT	TTC	AGC	
402																		447	
411																			
CTG	ATA	CAG	ATT	AAA	TCA	GAA	CGC	AGA	AGC	GGT	CTG	ATA	AAA	CAG	AAT	TTG	CCT	GGC	
459																		504	
468																			
477																			
486																			
495																			
504																			
GGC	AGT	AGC	GCG	GTG	GTC	CCA	CCT	GAC	CCC	ATG	CGG	AAC	TCA	GA					
516																			
525																			
534																			
543																			

FIG. 37

## Protein Sequence of TAP6H8 trcF

page 1

Length of TAP6H8 trcF: 751 bp; Listed from: 1 to: 444;  
 Translated from: 7 to: 427 (Entire region);  
 Genetic Code used: Universal; Mon. Aug 14, 2000 3:19 PM

Frame 1

M	A	H	H	H	H	H	H	Y	G	R	K	K	R	R	
NAG	ACC	ATG	GCT	CAT	CAC	CAT	CAC	CAC	TAT	GGC	CGC	AAA	AAA	CGC	CGT
9		18		27					36					45	

Q	R	R	R	M	N	A	L	Q	E	D	T	P	P	G	P	S	T	V
CAG	CGC	CGT	CGC	ATG	AAC	GEG	CTG	CAG	GAA	CAT	ACC	CCG	CCG	GGC	CCG	TCC	ACC	GTG
60		69						78				87				96		105

F	R	P	P	T	S	S	R	P	L	E	T	P	H	C	R	E	I	R
TTT	CGC	CCG	CCG	ACC	TCC	TCC	CGC	CCG	CTG	GAA	ACC	CCG	CAT	TGC	CGC	GAA	ATC	CGC
117					126			135		144				153			162	

I	G	I	A	G	I	T	I	T	L	S	L	C	G	C	A	N	A	R
ATC	GGC	ATC	GGC	GGC	ATC	ACC	ATC	ACC	CTG	TCC	CTG	TGC	GGC	TGC	GCG	AAC	GCG	CGC
174					183				192			201			210		219	

A	P	T	L	R	S	A	T	A	D	N	S	E	N	T	G	F	K	N
GCG	CCG	ACC	CTG	CGC	TCC	GGC	ACC	GGC	GAT	AAC	TCC	GAA	AAC	ACC	GGC	TTT	AAA	AAC
231					240				249		258				267		276	

V	P	D	L	R	T	D	Q	P	K	P	P	S	K	K	R	S	C	D
GTC	CCG	GAT	CTG	CGC	ACC	GAT	CAG	CCG	AAA	CGG	CCG	TCC	AAA	AAA	CGC	TCC	TGC	GAT
288					297			306			315				324		333	

P	S	E	Y	R	V	S	E	L	K	E	S	L	I	T	T	T	P	S
CCG	TCC	GAA	TAT	CGC	GTC	TCC	GAA	CTG	AAA	GAA	TCC	CTG	ATC	ACC	ACC	ACC	CCG	TCC
345					354			363			372			381		390		

R	P	R	T	A	R	R	C	I	R	L	.	.	.	.	.	.	.	.
CGC	CCG	CGC	ACC	GCC	CGC	CGC	TGC	ATC	CGC	CIC	TGA	AAG	CTT	GGC	TGT	TTT		
402				411			420			429				438				

FIG. 38

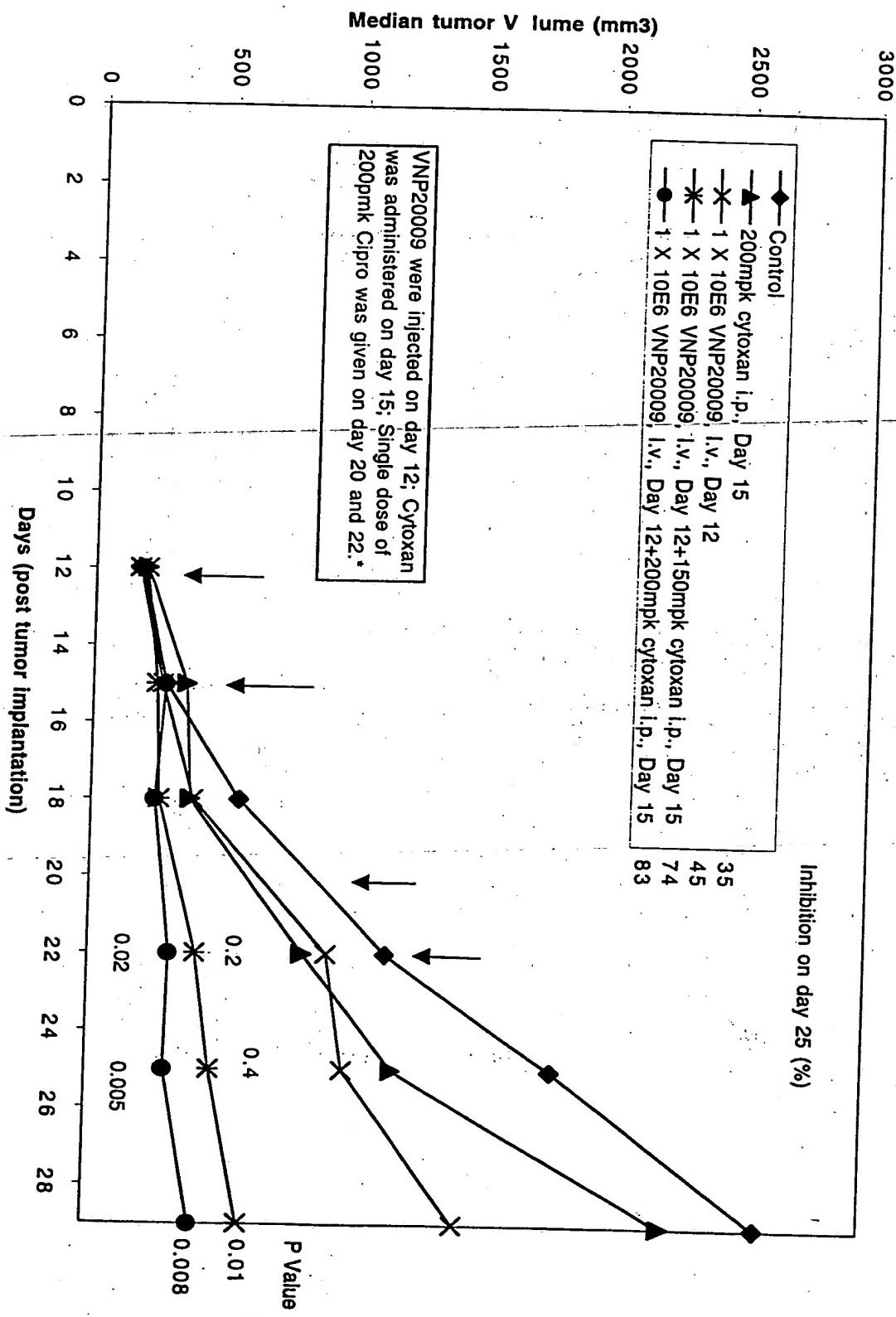


FIG. 39

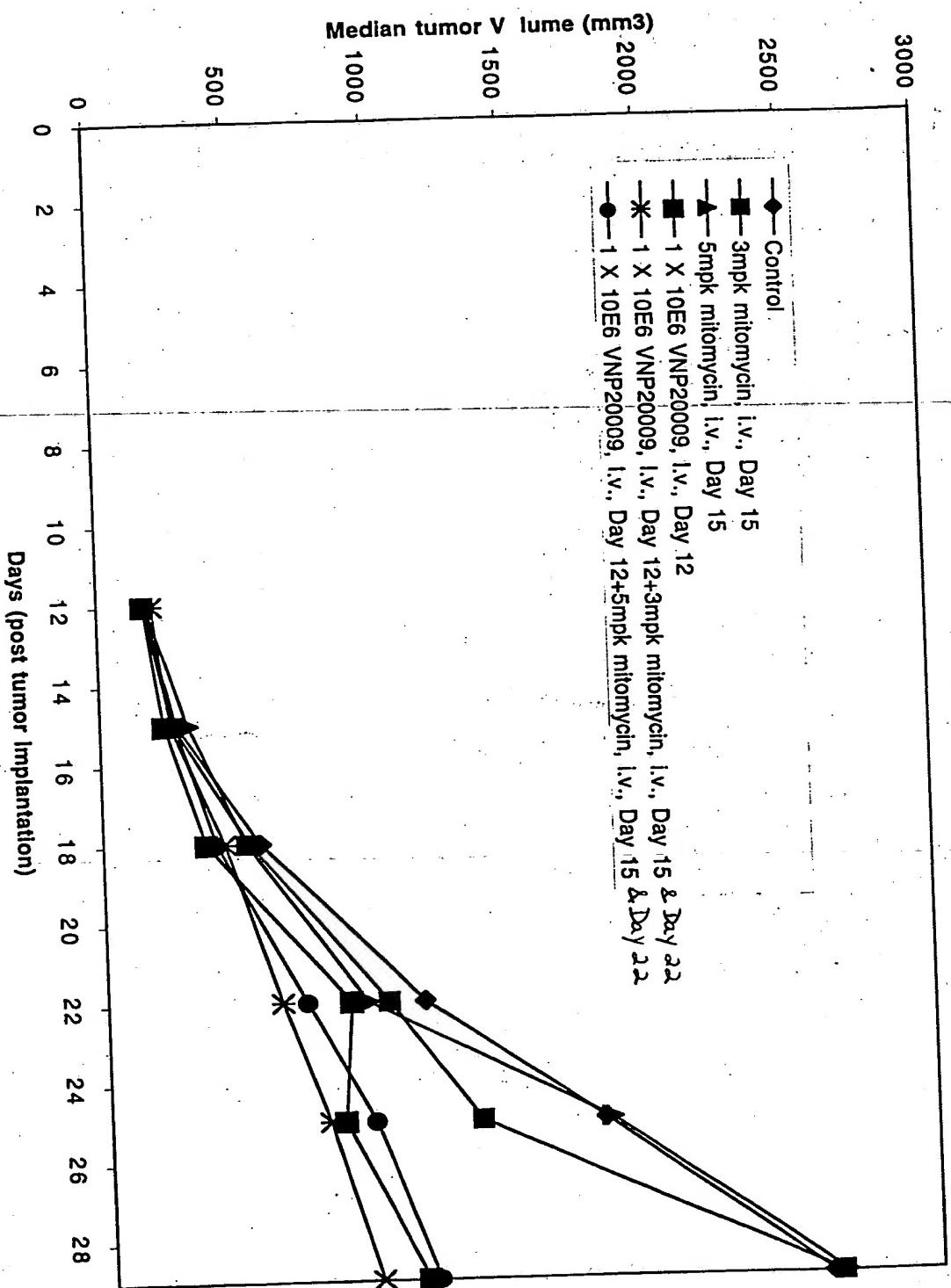


FIG. 40

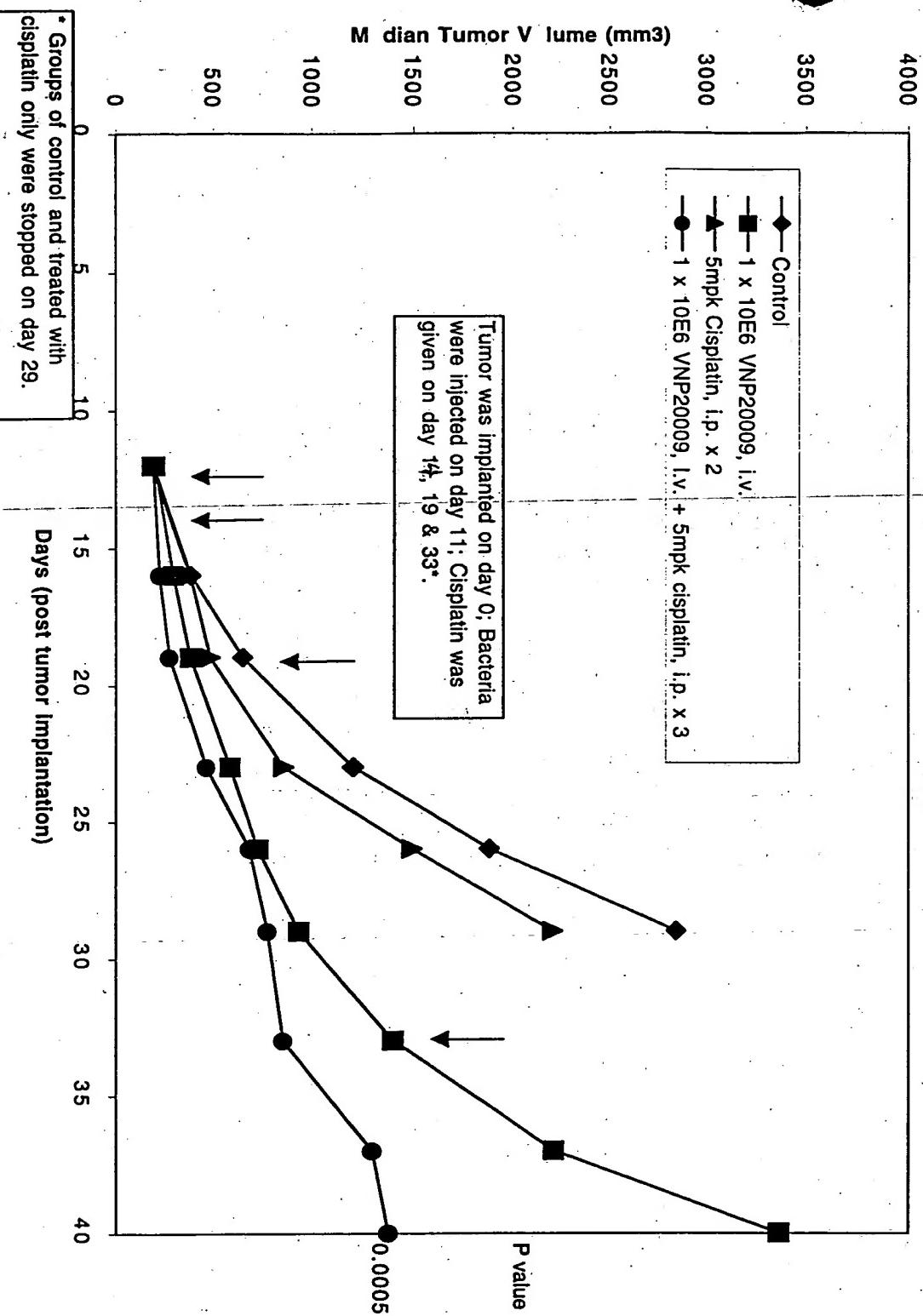


FIG. 41